DISPUTED POINTS AND UNSOLVED PROBLEMS IN THE NORMAL AND PATHOLOGICAL HISTOLOGY OF ENAMEL[1]

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INTRODUCTION

A little more than a year ago, as a result of the initiative and inspiration of Prof. William J. Gies, there was organized, here in New York City, the International Association for Dental Research. The conception of this organization was in full accord with the most advanced spirit of the times. Everybody, except the politicians, seems more or less aware that what we call civilization is entering on a new era in which coöperation for mutual benefits will take the place of competition for selfish ends. Science is largely responsible for this great forward movement, and science, the torch bearer of progress, will continue to lead the way.

Dentistry must contribute its share in this new order of things that is being established. If American dentistry has something to give Europe in methods of practical detail, Europe can bestow a full return

[1] Read at a meeting of the New York Section of the International Association for Dental Research, at the New York Academy of Medicine, January 27, 1922.

from her more thorough knowledge of scientific foundations and fundamental principles.

It is the hope of the founders of the International Association for Dental Research that not only will it serve to draw the whole dental world closer together in aims and ideals but it will also greatly harmonize methods of practice by stimulating original research work, the results of which will demonstrate what is truly scientific in our methods and what is not.

Branches of the organization have already been established in Boston, Toronto and Chicago, and it is the intention of the founders to establish branches gradually in all parts of the civilized world.

It requires no suggestion from me for anyone to see what a power for good in dentistry such a society may become if supported by a general sentiment and effort on the part of the profession—*and if we can keep dental politics out of it.* Such an organization assumes, at least at the outset of its career, a great responsibility. It must, by the work which it accomplishes, command acknowledgment of leadership in its own field. And there is crying need of leadership in dentistry as in every other department of the activities of modern life. It will be one of the main objects of this Society to seek out and to encourage in all possible ways men who give evidence of ability to advance the frontiers of truth in dental science.

Prof. Michael F. Guyer of the University of Wisconsin, in a recent address before the Wisconsin chapter of the Sigma Xi, said: "Human society has become so complex that no longer can its conduct be entrusted to the man in the street. It must, if it is not to prove the colossal failure of all time, be delegated to the expert." How great the need is in dentistry for such leadership and advice as the expert in original research work is alone fitted to give has been pointed out in an article in a recent issue of the Dental Cosmos, in very vigorous and incisive language, by Dr. Sidney R. Miller of the Johns Hopkins Medical School. Dr. Miller says: "No one can deny that current medical and dental literature contains an amazing chaotic mass of divergent opinion, diagnostic expedients and radical pseudoscientific propaganda."

While I feel that this is a rather severe indictment, I am, at the same time, aware that Dr. Miller could make a defense that would probably

humiliate us if his statement were attacked. To take only one point in this statement, is it not true that we have far more divergent opinion in our current literature than would appear if men were well grounded in the fundamentals of science? These divergences of view can be cleared up and settled only by original research work.

In accepting the office of chairman of the parent organization of this new Society I felt it my duty to take up for investigation some one of the problems about which there is pro and con discussion in our profession. And it seemed to me that one of the most important questions about which there is a wide divergence of opinion is that of possible chemical and metabolic changes in the enamel of erupted teeth. And so I have set myself the task of re-investigating this subject. I say "re-investigating" because, as most of you will remember, I gave considerable time to the study of this subject more than twenty years ago.

I expressed the opinion at that time that enamel contained no organic matter and that there could, therefore, be no question of organic changes in this tissue. This opinion was indorsed and maintained by the late Dr. Black up to the close of his life. This view has also been maintained by Sir Charles Tomes and many others. Prof. Hopewell Smith, in a remarkably able and vigorous address given in London last July, claims that enamel is a dead tissue. To a very considerable extent this opinion has been based on the failure of efforts to stain normal human enamel.[2] But more recently a number of investigators in this field, including so accomplished an expert as Mr. J. Howard Mummery of London, have announced their success in staining human enamel.

In addition to this evidence of the presence of stainable matter in enamel there has, for some time, been a growing body of opinion in the profession, based on clinical observation, that changes take place in enamel which must be due to chemical or metabolic action. This opinion has been greatly strengthened by the highly accomplished scientific work of Professor Pickerill.

[2] In an article published in 1916 Dr. Black said: "We cannot stain the body of normal enamel with any staining agent we know. Even the anilin dyes dissolved in alcohol fail to penetrate it." In this same paper he also said: "The tissues of the teeth are not changed in any way by physiological processes after they are once formed."

Now, obviously, there is no more important problem before our profession today than this. If it could be conclusively demonstrated that metabolic, or even so-called inorganic, chemical changes take place in the enamel of erupted teeth, the door would be open for investigations which might result in the discovery that such chemical changes could be controlled in a manner to improve the quality or resisting power to decay of that tissue. That would indeed be a momentous day in the history of dentistry and, I may add, in the history of humanity. On the other hand, if there be a considerable body of opinion in the profession with views and beliefs as to the possibility of enamel regeneration which have no foundation in fact, then, obviously, the profession is, just to the extent of this body of unfounded opinion, handicapped in its efforts to find out if by any means the resistive power of this tissue to decay may be improved. The question, you see, really involves the whole problem of the prevention of decay.

And so, last May, I began the investigation of this subject. New and important facts have come to light but I am not yet ready to make any report on the main question. Much more work remains to be done before a definite conclusion can be reached. I will only say at this time that I am very hopeful that such a conclusion will be reached.

In the meantime this investigation has cleared up a number of long-standing problems most of which have a more or less direct bearing on the main question. Because of the bearing of these points on the main issue, and also because of the intrinsic interest in the points for and of themselves, I have thought best to make this present preliminary report.

The various subjects treated are not as disconnected as might at first appear. They all lead directly up to the main question, which is this: is human enamel, in the average tooth as we find it to day, sufficiently porous to admit of some sort of circulation of possible nutrient fluids? And the subsidiary question is: what bearing do all the facts in this respect, now in our possession, have on degenerate and pathologic forms of enamel?

THE STRUCTURE OF MARSUPIAL ENAMEL

The most penetrating study of structure must precede a comprehension of normal or abnormal functions or conditions. As you are all aware, it has many times happened in scientific work of this character that much light has been thrown on problems of structure and function in human tissues and organs by the study of the structure and function of corresponding parts in the lower animals. I have no wish to overemphasize the significance of phylogeny in this respect but there is no possibility of escaping the fact that the roots of every phase of the structure of the tissues of the human body are to be found deep in the lower forms of life which preceded man. If, therefore, we find tubularity of enamel a not uncommon condition in many lower forms of life we shall, naturally, not be surprised to find that it sometimes occurs in human teeth. But whether this is to be considered merely as something exceptional without special implication of degeneracy or as evidence of reversion to a lower type is another question.

There is something very vague about this expression "reversion to a lower type." We are by no means sure what we mean when we use this expression. There is much more organic matter in the enamel of the teeth of some animals which never suffer from caries than in human enamel. We are not yet certain that the hardest enamel is the most resistent to decay. It may be so but it has never been demonstrated to be a fact. Furthermore, softness or hardness of enamel does not necessarily imply more or less of organic matter. But quite apart from these somewhat speculative matters there is no doubt but that Charles Tomes placed great significance on the structure and method of formation of marsupial enamel as explaining the method of formation and structure of human enamel. That there is considerable organic matter, in the shape of uncalcified fibers, in marsupial enamel has long been known.

According to Tomes's view these fibers occupy the center of the enamel rods. His view is that calcification of the enamel rod begins at the periphery and proceeds toward the center. Sometimes the

calcification of the entire rod is completed but in many instances it is not. A small fiber, closely corresponding in size to the dentinal fiber, is left as the uncalcified center of the enamel rod. In the teeth of some of the fishes there are widely varying systems of tubes and fibers. I say "tubes *and* fibers" because, obviously, not all of the tubes found in enamel contain fibers of living or organic matter. For illustration, the teeth of the Sargus have tubes which are of considerable diameter on the surface of the enamel. These tubes gradually diminish in size as they pass into the substance of the enamel and apparently disappear completely when they have penetrated about two thirds of the thickness of the enamel. It is hardly to be conceived that these tubes contain living matter. On the other hand we find, in many of the fishes, a system of tubes and fibers penetrating the enamel from the dentine. These fibers are prolongations of the dentinal fibrillae.

Living, organic matter which is functionless probably does not exist. Wherever organic matter is found, there metabolic changes are taking place. When, therefore, we find an abundance of organic fibers in marsupial and other forms of enamel, we are well justified in assuming that these fibers are performing some of the functions of living matter. And if it could be shown that human enamel is, as claimed by Tomes, formed in much the same manner as marsupial enamel and if it could be demonstrated that any portion of uncalcified matter remained, we should be justified in assuming that here also organic changes were taking place. You see, therefore, that a critical examination of the formation and structure of marsupial enamel becomes a very important matter—from the Tomes point of view.

In 1905 I showed Tomes some photographs of transverse sections of the enamel of the kangaroo in which many of the fibers were clearly seen to be in the cement substance *between* the enamel rods. In the seventh edition of his "Manual of Dental Anatomy" he mentions these photographs in the following words:

> Leon Williams has photographed some transverse sections of marsupial enamel, in which three fourths of the tubes appear clearly to be in the substance of the rods. The remaining fourth appear as though they were between them. But it is à priori highly improbable that they occupy

both positions, and a position within the rod is much more readily reconcilable with the appearances seen in the development of marsupial enamel.

It is clearly seen that Tomes, in this statement, is casting doubt on the apparent evidence of the photographs that some of the fibers are in the cement substance. Also I think Tomes largely overestimated the number of fibers that appeared to be *in* the enamel rods.

Elsewhere in the same volume, in speaking of the ratio of fibers to rods, he says:

> In the finished enamel the majority of the rods are solid, as may be seen in Leon Williams's beautiful photographs of transverse sections of kangaroo enamel. . . . . On the whole a tube occurs in about one out of every four rods of the kangaroo.

Tomes' view of the bearing of this on the structure of human enamel is clearly seen in the following passage from the work already quoted:

> But if tubularity is a stage through which every enamel rod passes, the difficulty of accounting for animals, whose allies have lost it, re-acquiring or developing it, disappears. And although the examination of human or other solid-developing enamels might never have led to this interpretation, yet a re-examination of them, after having arrived at it, convinces the observer that it is equally true of all.

Mr. Tomes is here dealing with a generalization which would be very important if it could be established.

Mr. J. Howard Mummery saw the photographs to which Tomes refers and later made a very careful and thorough investigation of the subject. He reached a conclusion, published in his "Microscopic Anatomy of the Teeth" in 1919, exactly diametrical to that of Tomes. In his opinion, although an occasional tube or fiber may *appear* to be in the rod, "any appearance of its so doing may probably be fully explained as being due to the thickness of the section." It is rather curious to find these two highly trained observers taking exactly opposite views and each insisting that it is highly improbable that the tubes or fibers should appear in both rods and cement substance. It should be noticed, in passing, that both Paul and Von Ebner agree with Mummery that the enamel fibers are in the cement substance.

The explanation of the disagreement among a number of the highest

authorities in this field must, I think, be wholly attributed to a lack of convincingness of their preparations and photo-micrographs. While it is of course true that a considerable amount of training is necessary for the correct interpretation of photo-micrographs in any special field of study, it is also emphatically true that, other things being equal, those photographs which show the most detail of structure will yield the most information. I long ago pointed out that the so-called classic balsam method of preparing and mounting specimens was about the poorest for the study of histology of the dental tissues. I shall call your attention this evening to a considerable number of new facts in the structure of enamel. Very few of the photographs exhibiting these new facts were taken from balsam mounted specimens.

We will now proceed to an examination of some of these studies of marsupial enamel. It is exceedingly difficult to get a good and well stained transverse section of kangaroo enamel. One reason for this will be clear from the photograph now on the screen (*fig. 1*) which shows a transverse section of the lower incisor of the large grey kangaroo (Macropus giganteus). The many abrupt changes in direction of the enamel rods make a cross section of those rods a very elusive thing. I have ground more than 100 sections of kangaroo enamel and have succeeded in only a few specimens in getting completely convincing evidence on the point under consideration.

There are two totally distinct phases of this problem to which I wish to call your attention. (*a*) Are the enamel fibers in marsupial enamel a strictly enamel production as claimed by Tomes or do they have their origin in the dentine as I suggested more than twenty-five years ago? (*b*) Do these fibers pass in the rods or in the cement substance or in both?

Tomes, while admitting that the enamel fibers are connected at the dento-enamel border with the dentinal fibers, claims that this connection is in the nature of an anastomosis (*fig. 2*). The fact that in many instances there is a distinct enlargement or knot-like appearance at the dento-enamel junction of these fibers gave considerable color to Tomes's claims. It is also to be observed that in many instances this knot-like enlargement does not appear but the fibers pass without change of size directly from the dentine into the enamel (*fig. 3*). The final proof that the enamel fibers are continuations of the dentinal

FIG. 1. Section of incisor of large grey kangaroo. In trying to understand the form-arrangement of the enamel rods in the teeth of any animal it should always be kept clearly in mind that a section represents but a *single* plane while the real form-arrangement is in *three* planes. The spiral represents the typal course of the enamel rods in human teeth and the teeth of most animals. If we were to take a corkscrew with a solid core and grind a thin longitudinal section to the center, the outline of the figure would closely resemble the arrangement of the enamel rods in the above illustration. Sections of enamel must be ground at many angles in order to comprehend the ultimate arrangement of the rods.

fibers came in a fortunate specimen ground from our American marsupial, the opossum. You have on the screen (*fig. 4*) a photograph of a longitudinal section of a lower premolar of this animal. We at once observe certain peculiarities in this specimen and these peculiarities solve one of our problems. You observe that the dentinal fibers are in groups, some appearing dark and others light. This is an

FIG. 2. From tooth of Kangaroo. *A*, dento-enamel border. At *A* fibers are seen emerging from the dentine and passing into the enamel. At *B* is seen the branching of a fiber. Although the normal position of these fibers in the enamel is in the cement substance, when the fibers branch, as shown in this illustration, during the development of the enamel and while the tissue is still soft, they may pass into and through an enamel rod. If, therefore, a section happens to be cut just where a fiber is in the rod we have the explanation of the Tomes theory that these fibers represent the uncalcified portion of the center of the enamel rod.

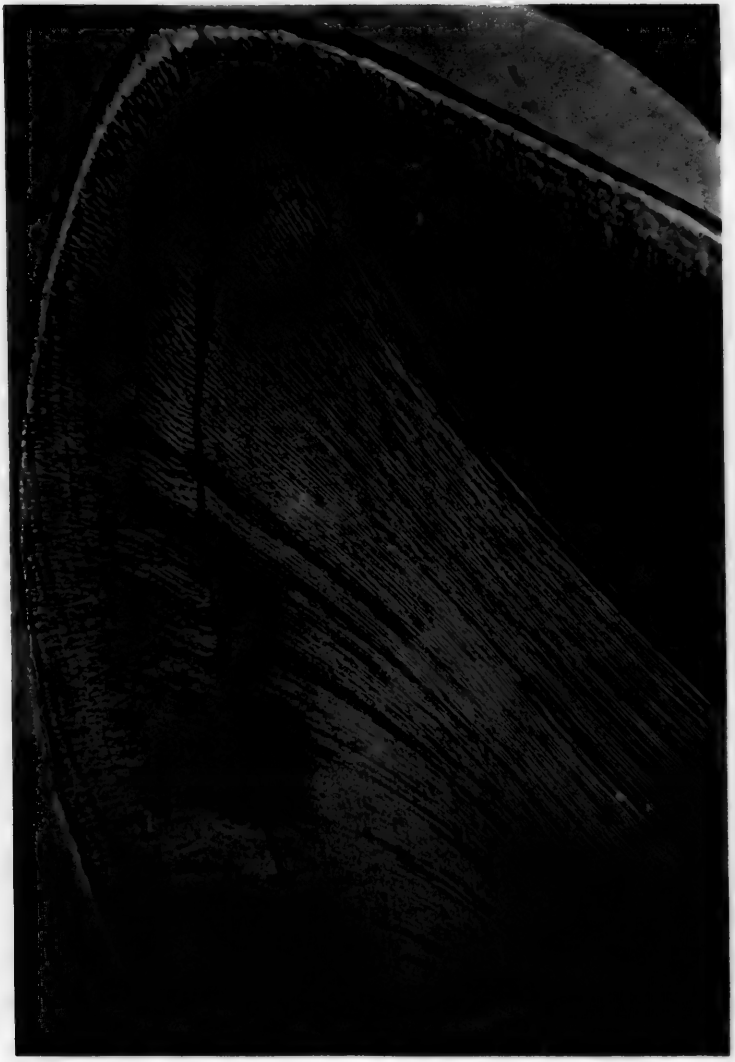


FIG. 3. Section from lower pre-molar of opossum, the only American marsupial. The chief characteristic in the minute anatomy of the teeth of the marsupials is the free passage of the dentinal fibers into the enamel. A few of these fibers penetrate the entire thickness of the enamel but the majority do not reach the surface.

Tomes has always contended that these fibers are not continuations of the dentinal fibrillae but are formed in the enamel by the non-calcification of the centers of the enamel rods. In the view herewith presented it is seen that wherever there are dark colored groups of fibers in the dentine these groups also appear as dark colored fibers in groups in the enamel. This view, therefore, presents the final and complete proof that these fibers are of dentinal origin. The exact position of these fibers in the enamel will be shown in following views.

appearance often seen but the significance here is much greater than usual. For, observe, wherever you see a group of the dark-appearing fibers in the dentine, that group is also seen, continued straight on into the enamel. This is true of all the groups and all the individual fibers.

FIG. 4. Section from the lower pre-molar of the opossum, the only American marsupial. A portion of *fig. 3* under a higher power. Nearly all of the marsupials have organic fibers in the enamel. The origin of these fibers has long been a subject of controversy. This illustration settles the dispute by showing clearly that the fibers in the enamel are prolongations of the dentinal fibers.

One familiar with the law of probability could tell us what the chances might be that all of those groups of fibers would have a separate origin, one being in the dentine and the other in the enamel, and that they all united at the dento-enamel border at exactly the right spot. But I suppose the chances that this might occur would not be one in ten millions, and we may therefore safely conclude that the enamel fibers in marsupial enamel are prolongations of the dentinal fibers.

Let us now examine the position of these fibers in their relation to the enamel rods. The photograph on the screen (*fig. 5*) is from a transverse section of enamel from the upper incisor of a kangaroo. Some of the enamel rods are shown in exact transverse section and others are cut somewhat obliquely. The organic fibers are deeply stained and show very plainly on the unstained background of the enamel. This is a good general view of a large field under a comparatively low magnifying power. To see clearly the relation of the organic fibers to the enamel rods we must use higher powers.

The succeeding photographs (*figs. 6, 7, 8,*) are all from sections of kangaroo enamel and tell the whole story of the structure of marsupial enamel.

In all of the many specimens I have examined I have in only two or three instances found the fiber in the center of an enamel rod. In a small percentage of instances the fiber occupies varying positions in the rods, mostly at the sides. At least 90 per cent of the fibers are in the cement substance between the rods (*fig. 9*). We see, therefore, that both Tomes and Mummery are wrong; the one in claiming that *all* of the fibers are in the enamel rods and the other in placing them *all* in the cement substance.

It would seem that the normal position of the fibers is in the cement substance, but a few of them divide in the enamel (*fig. 2* at B), and a few others abruptly change direction without reference to the course of the enamel rods (*fig. 4*). Some of these erratic fibers enter the substance of the enamel rods while they are still soft and it was undoubtedly this circumstance which misled Tomes. But the evidence which I have presented this evening that the great majority of the organic fibers in marsupial enamel are in the cement substance is so clear and unmistakable as to leave no possible room for doubt,

and we may now regard this longstanding problem as solved and settled. But the function of these organic fibers in marsupial enamel is still a mystery.

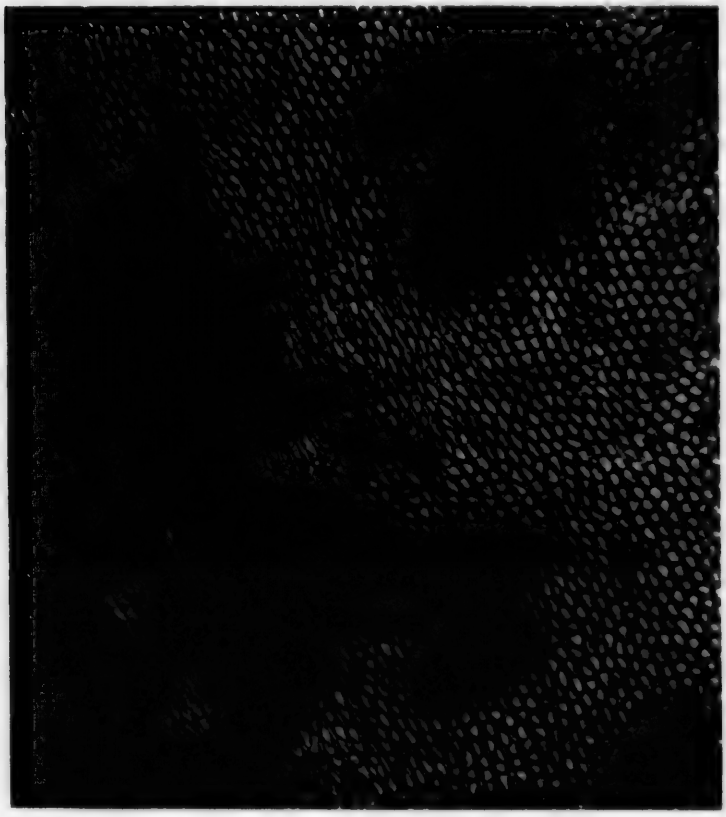


FIG. 5. Section of incisor of Kangaroo, magnified about 300 diameters. Stained with nitrate of silver solution. Shows the enamel fibers stained black.

WHAT IS THE ENAMEL ORGAN?

The caption with which I introduce this section of my paper may have aroused some little surprise in the minds of dental histologists. "Is there any doubt," they would remark, "as to what constitutes the enamel organ?" Let me confess that I have used this particular

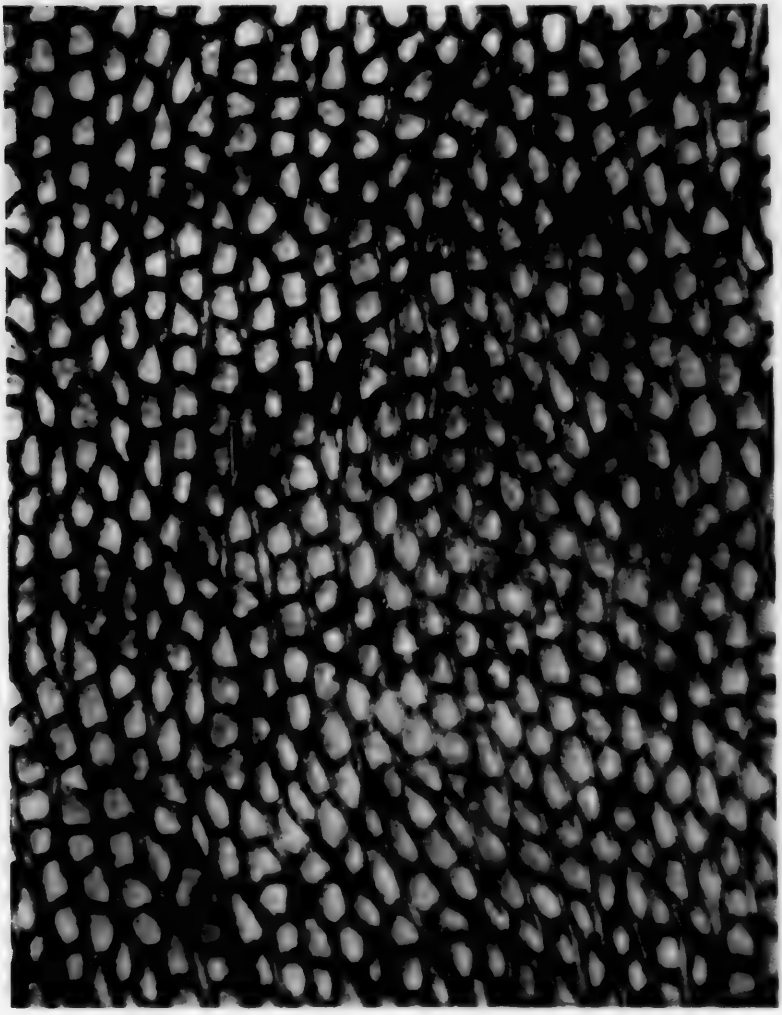


FIG. 6. Section of tooth of Kangaroo magnified about 1,000 diameters. The tubes in which the fibers pass are seen to be largely in the cement substance between the enamel rods, sometimes encroaching somewhat on the substance of the rod. Nitrate of silver stain.

form of caption to call attention to what I regard as a long-standing error concerning the functions and relative significance and importance of the several features or parts of the enamel organ. When the germ of a human tooth is completely encapsuled, the enamel organ consists of the following named several parts. First, there are the

FIG. 7. A cross or transverse section of *fig. 1* under a magnifying power of about 1000 diameters, stained with a solution of nitrate of silver. This shows that the organic fibers are mostly arranged in groups and that their position is nearly always in the cement substance between the enamel rods. A few, however, as at *A* and *B*, are seen in the enamel rods. These fibers penetrate the enamel during the early stages of the formative period before calcification begins.

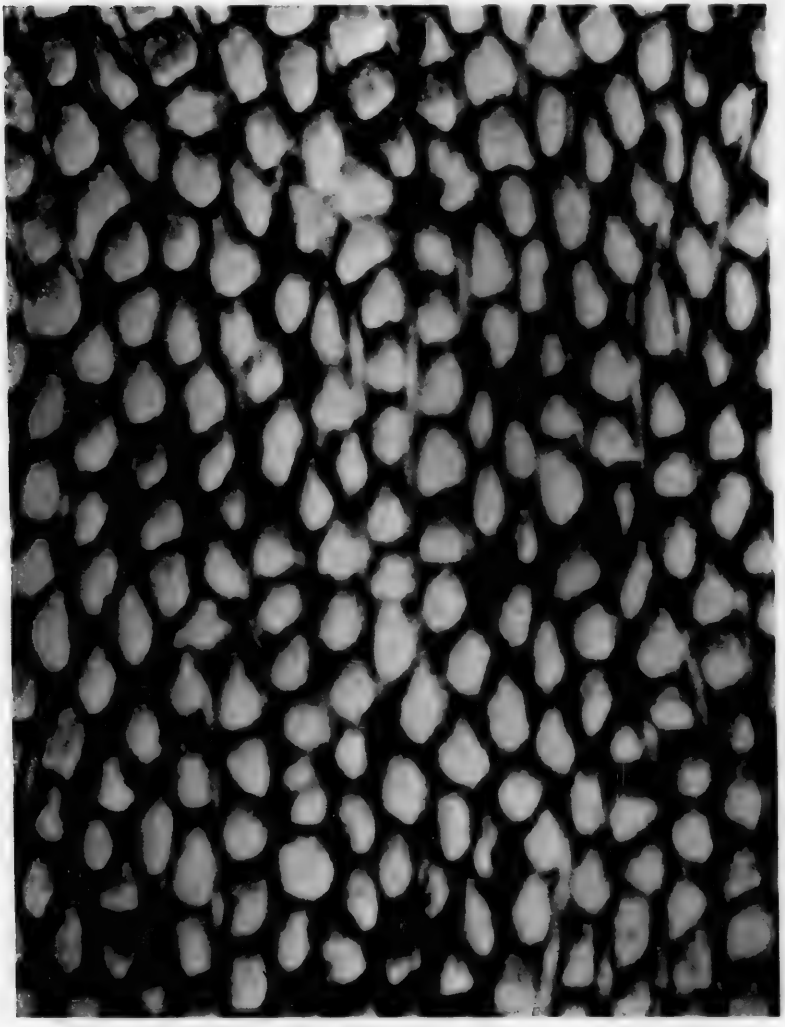


FIG. 8. This transverse section of the enamel of the incisor of the Kangaroo, magnified about 1,200 diameters, was focused on the extreme ends of the enamel rods so as to clearly demonstrate that not a single fiber can be seen emerging from an enamel rod while an abundance of the tubes are everywhere seen in the cement substance between the rods. This group of photomicrographs completely settles the long disputed question of the origin and position of the fibers in the enamel of the marsupials.

FIG. 9. This view shows that the fibers in marsupial enamel are usually more or less in groups, sometimes a dozen or more being seen in close proximity together with considerable areas of enamel in which no fibers appear.

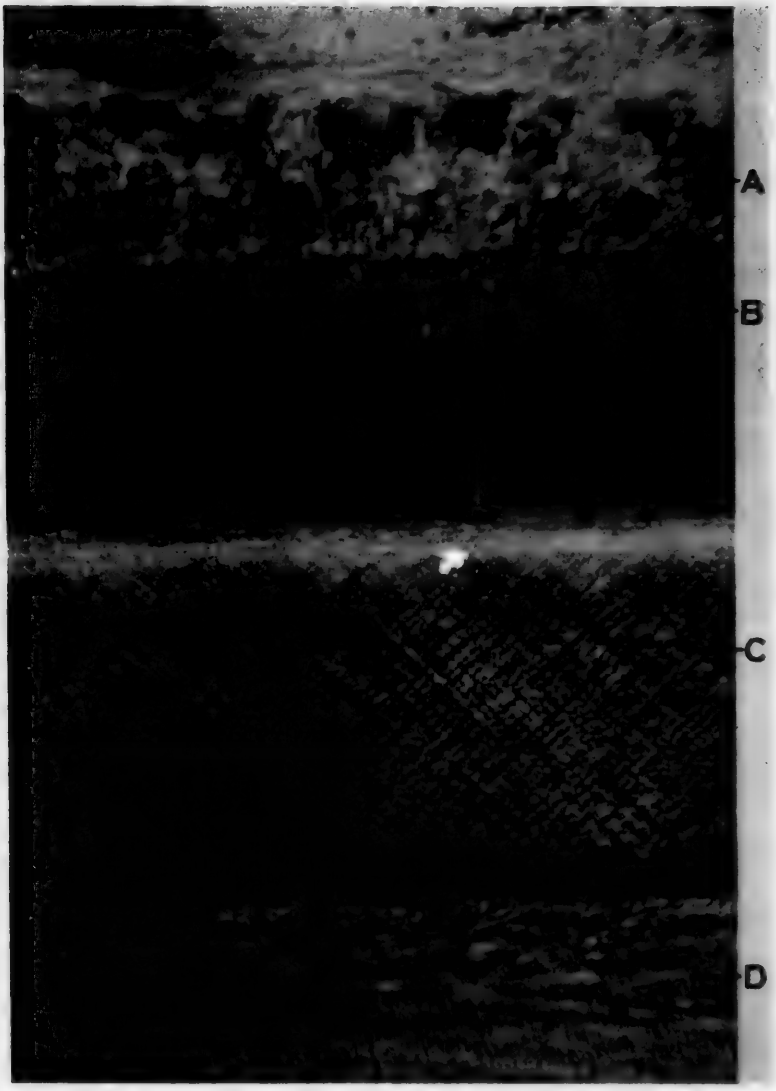


FIG. 10. Formation of enamel in the persistently growing incisor of the albino rat. It is seen that the cells of the stratum intermedium, shown at *A*, have been converted into a secreting organ with many papillae arranged around the blood vessels which are shown dark in cross section. The staining in this subject was effected by the injection of Trypan blue into the abdominal cavity. All of the tissues except the fully formed enamel are stained. *B*, Ameloblasts; *C*, Enamel; *D*, Dentine.

ameloblasts, the cells which give form to the enamel rods. On the outer ends of the ameloblasts lie two or three layers of round or oval-shaped cells known as the *stratum intermedium*. Next comes the *stellate reticulum* which, in the early stages of the tooth germ, occupies much the larger part of the tooth sac, but which rapidly decreases in size and, in many instances, disappears long before the formation of enamel is completed. The outer part of the stellate reticulum is completely covered by a layer of epithelial cells which in their origin are identical with the cells of the stratum intermedium. This outer layer of epithelial cells is covered by connective tissue in which the blood vessels supplying the enamel organ are first developed.

Now, there has been a great deal of discussion as to whether blood vessels are ever seen in the enamel organ, and nearly all the writers who have contributed to this discussion mean "stellate reticulum" when they use the term "enamel organ." In other words, they use these two terms as meaning the same thing.

One of the latest articles on this subject that I have seen is by Dr. H. E. Jordan of the Department of Histology and Embryology, Medical School, University of Virginia. The title of Dr. Jordan's paper, which appears in *The American Journal of Anatomy* for September, 1921, is "The comparative histology of the enamel organ of the mammalian tooth, with special reference to its blood supply." Dr. Jordan says:

> The extensive network of extrinsic capillaries in their ramification over the surface of the enamel organ, and pressing upon the outer tunic, becomes so intimately blended at certain points with the outer enamel epithelium that the endothelium and the enamel cells become fused into a continuous membrane. . . . . But neither the capillary wall itself nor the capillary lumen as such is continued into the enamel organ.

By "the enamel organ" Dr. Jordan obviously means the stellate reticulum.

A little later in this article Dr. Jordan credits me with having been the first to call attention to the formation of a papillary layer of cells in the enamel organ and its close resemblance to secreting tissue. Then, referring to a paper which I read before the Royal Society of Great Britain in 1895, he says: "Williams accordingly derives the

papillae of the papillary layer from the cells of the stratum intermedium." Now, this is exactly what Williams did not do; on the contrary, he tried to make it as plain as his command of the English language would allow that the formation of the papillary layer began in the outer epithelial layer of cells.

Here is the exact language as published in the Dental Cosmos in 1897.

> The outer surface of the stellate reticulum is bounded by epithelial cells identical in character with those from which the stratum intermedium is developed. With the disappearance of the stellate reticulum these two layers come together and unite. But often before this takes place the secreting papillae have already commenced to form about capillary loops in the *outer layer* of epithelial cells already mentioned as covering the stellate reticulum. It is possible, and, I think, probable, that the evolution of the secreting papillae always *begins* in the outer layer, but it is perfectly evident in many of my specimens that after the stellate reticulum disappears and the outer layer of epithelial cells with the forming blood vessels comes in contact with the stratum intermedium, the cells of both layers are converted into secreting papillae.

In view of such a clear and explicit statement I can not understand how Dr. Jordan could have so misrepresented my position.

But this matter of secreting tissue as a prominent feature of the enamel organ is, from my point of view, of such vital importance, with reference both to the structure of enamel and to its method of formation, that I ask your indulgence for a little closer examination of the whole subject. So long as merely general statements are made about enamel structure and photographs shown which do no more than illustrate these very general statements, the points to which I am about to call your attention are not important simply because the facts on which they are based are unseen and unknown. But when the finer structure of the enamel rod is revealed and when we consider the action of acids on enamel and several other important and unsolved problems with which we are now face to face, we see the necessity for new and improved methods of technique, for the greatest resolving power of lenses and the most critical study of the details which improved methods and apparatus reveal.

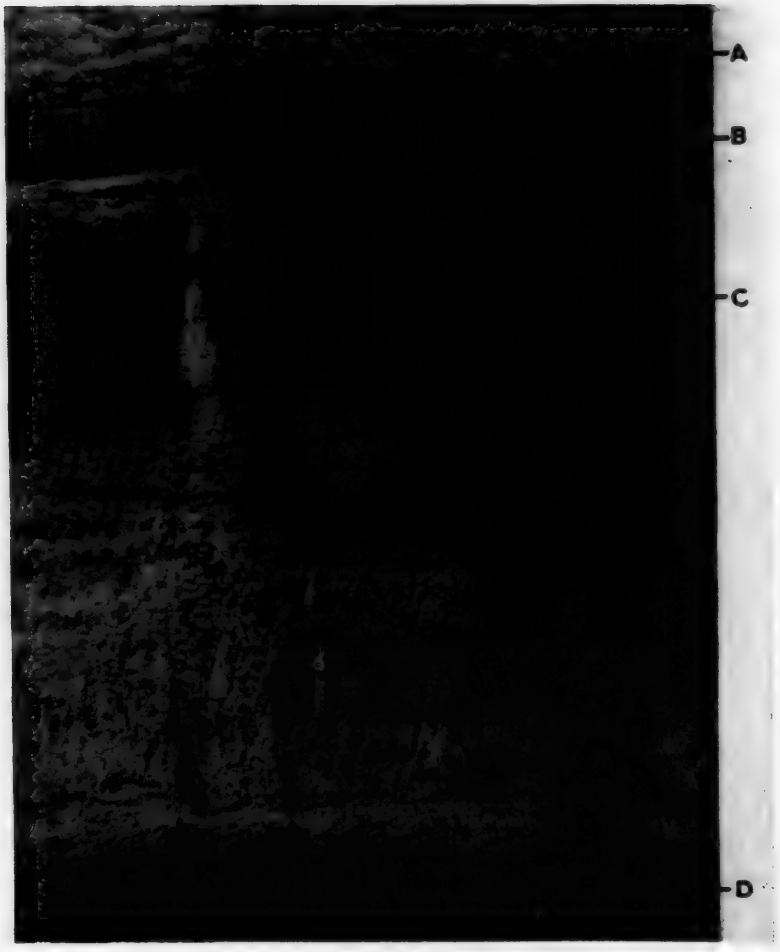


FIG. 11. Formation of enamel in persistently growing incisor of woodchuck. Injected and prepared the same as in figure 10. *A*, bloodvessel in secreting enamel organ; *B*, ameloblasts; *C*, forming enamel deeply stained with injected Trypan blue; *D*, dentine, also stained.

Dr. Jordan attempts to minimize the significance of the papillary or secreting layer of the enamel organ in the following statement:

> From the description of the comparative histology of the enamel organ of the incisors of the rat and of the cat it is clear that the so-called papillary layer is not the same thing in both cases. It is, however, formed in exactly the same way and through the operation of exactly the same factors: pressure upon the outer surface of the enamel organ by impinging capillaries. . . . . It never involves the stratum intermedium, except, in an indirect way, in the later stages of development when the stellate reticulum has disappeared and the ameloblasts have practically ceased functioning. . . . . Accordingly, contrary to the view of Williams and Skillen, the papillary layer has no specific significance in amelogenesis distinct from the extra-ameloblastic portion of the enamel organ as a whole.

There are two or three statements in this quotation which I do not think Dr. Jordan can successfully defend. It is not true, according to the facts presented in all the work that I have done, that the papillary layers in the cat and the rat are formed in the same way. It is not true that this layer does not appear until the ameloblasts have practically ceased functioning. And I do not think that any embryologist of reputation would agree with Dr. Jordan that the development of any tissue can be explained in quite so simple a manner as by his formula of "pressure upon the outer surface of the enamel organ by impinging capillaries." The actions, reactions and interactions which are always taking place in all growth and development are probably numerous and complicated beyond the power of any imagination to conceive.

Let me here mention a criticism on my views of this subject by Sir Charles Tomes and then further consider the strictures of both Tomes and Jordan together, as they both refer to practically the same points.

Tomes, in the seventh edition of his "Manual of Dental Anatomy," page 179, says:

> Leon Williams, seeing this large development of cells of the stratum intermedium distributed over vascular papilliform shapes, has attributed to the cells a glandular function, and sets down to them a very large share in enamel formation in the way of elaborating products for calcification.

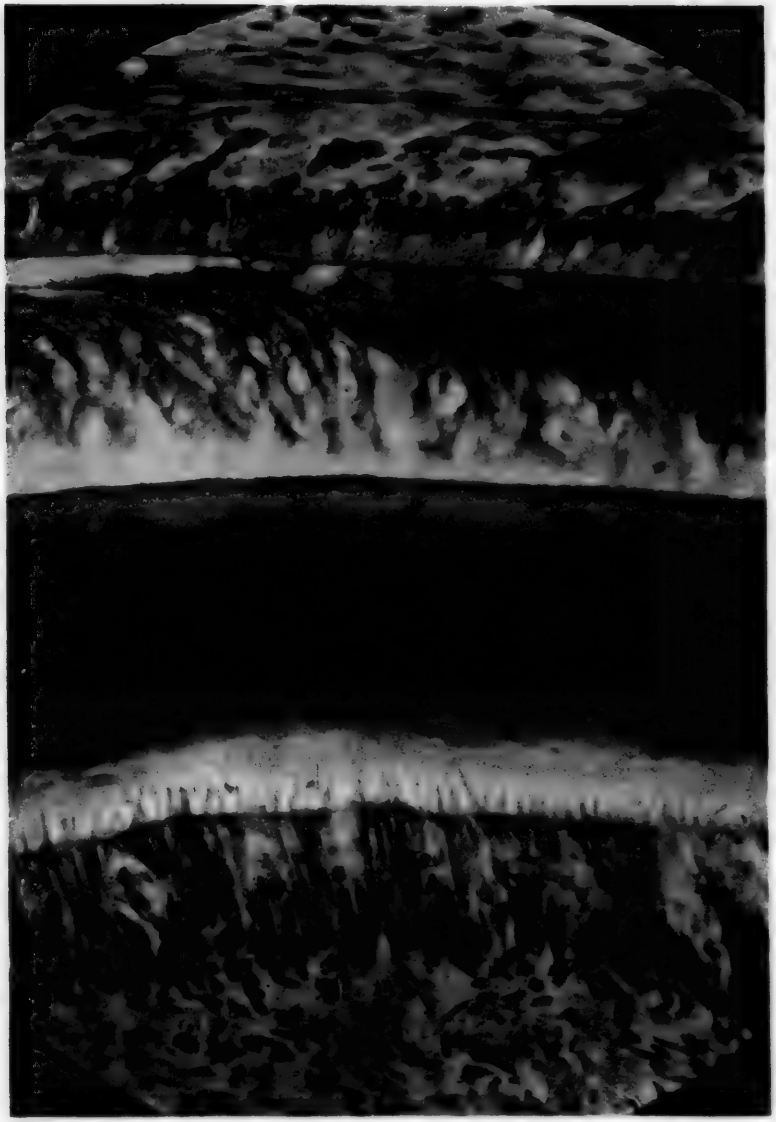


FIG. 12. Formation of enamel in persistently growing molar of rabbit. Here the secreting enamel cells are seen, above the ameloblasts, arranged in regular loops about the bloodvessels. There is never any trace here of a stellate reticulum and all of the elements of the enamel organ are developed nearly simultaneously, *in situ*.

But this is hardly a safe inference. In the first place this particular structure does not arise till after some enamel is already formed, and there is no known difference between the enamel formed before and after its development; moreover, it is only in some animals that it is at all well marked, and their enamel is not different in any known way from those that do not possess it.

That this statement is not sound reasoning is easily shown by substituting the term "stellate reticulum" for "papilliform layer" and then paraphrasing the whole passage. So treated it would read:

Many observers, seeing the large space occupied by the *stellate reticulum*, have attributed to this tissue a very important share in the process of enamel formation. But this is hardly a safe inference. In the first place this particular structure often disappears long before the completion of enamel formation; moreover, in some animals it never appears and their enamel is not different in any known way from those which do not possess it.

Thus, you see, by substituting the right term for the wrong one, we convert Tomes' own language into a perfectly logical statement which completely upsets his own contention. But let us have done with verbal fencing and get right down to the plain facts of the whole matter as revealed by the microscope.

The best authorities define secretion as the process by which material is separated from the blood by the agency of the cells of various glands, and by these cells elaborated into new substances. Certain substances are required for the formation of the enamel of a developing tooth. The material for one of these substances (that which contains the calcific matter) must come from the blood. The ameloblasts do not lie in direct contact with the blood vessels. The cells of the *stratum intermedium*, after the disappearance of the stellate reticulum, unite with the cells of the outer layer of the enamel organ and arrange themselves about capillary loops, after the manner of recognized secreting cells. *The material for the development of enamel, therefore, must pass through these cells before it reaches the ameloblasts.* Are we not then in a position to say that these cells select from the blood special substances for a special purpose? And does not this essentially constitute the function of secretion?

But this logical statement is only a part, and the lesser part, of the argument. There is a large class of animals, the rodents, with persistently growing teeth in which enamel is constantly being formed

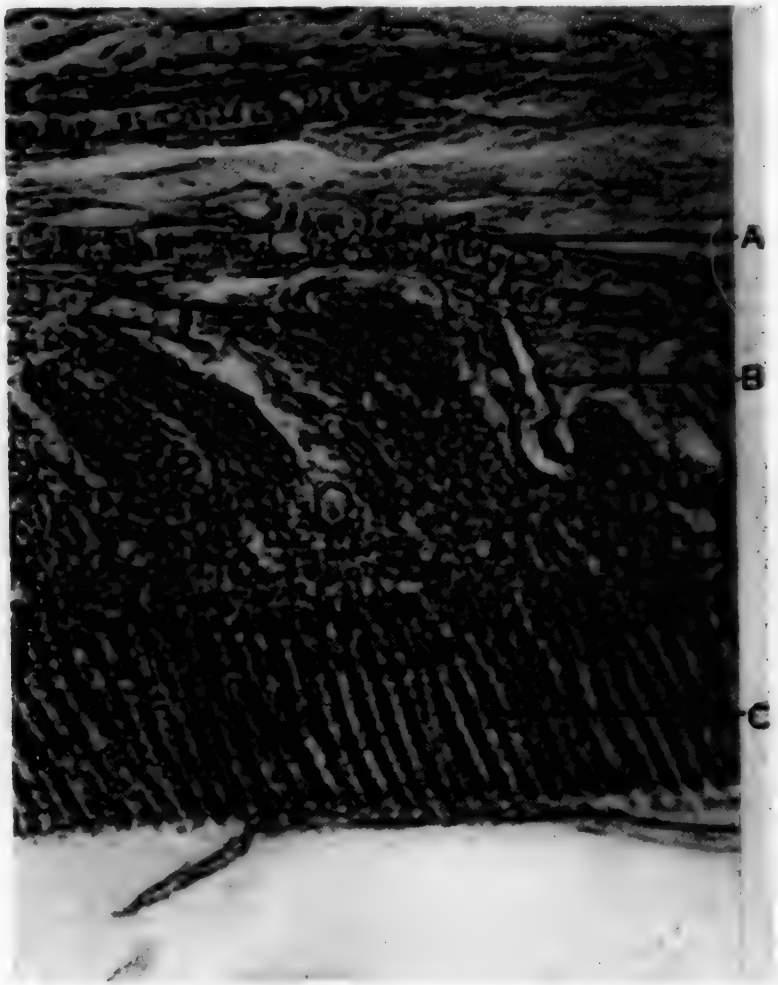


FIG. 13. Secreting enamel organ of rat. *A* and *B*, bloodvessels passing between secreting papillae; *C*, ameloblasts. This and the three following illustrations are from a former article in the *Dental Cosmos*.

throughout the entire life of the animal. According to Dr. Marshall's statement in his recently published articles in the JOURNAL FOR DENTAL RESEARCH the entire length of the lower incisor of the albino rat is changed every thirteen or fourteen weeks. This means very rapid formation of enamel. Now, the enamel of the rodents is considered of the highest order of perfection. And in none of the persistently growing incisors or molars of these animals is there ever to be seen any trace of a stellate reticulum. Nor is there ever any joining up of an outer with an inner epithelial layer of cells to form the enamel organ. The enamel organ is developed as a secreting organ, *in situ*, from the beginning. I have, during the past year, studied the persistently growing teeth of all the New England rodents, including the beaver, porcupine, woodchuck, muskrat and several varieties of rats, mice and squirrels, and the above stated facts are true of all these animals: their enamel organs contain no stellate reticulum.

But this is not quite all of the argument. I first made the statement nearly thirty years ago that the enamel organ proper is a secreting organ. Tomes criticised this view as I have pointed out. There was no confirmation or support given my view for more than twenty years. Then J. Howard Mummery read a paper before the Royal Society of Great Britain in which he completely confirmed my work. This confirmation is repeated and somewhat elaborated in his book "The Microscopic Anatomy of the Teeth," published two years ago. Mr. Mummery discusses this question at considerable length. I select the following passages as bearing directly on the points I am here considering.

"The appearance of a tubular secreting structure in the enamel organ seems to mark the later stages of calcification in *Sargus*, but in *Labrus* we find at very early stages indications of such a structure." Again, "The process of enamel formation would appear to be one of true secretion." Mr. Mummery gives me credit for having first called attention to the glandular nature of the enamel organ in the rat and adds: "His description and photographs certainly appear to indicate that at all events in the rat there is a survival of the secretory apparatus so highly developed in the fishes."

But there is further important evidence on the point. Both Tomes and Mummery have pointed out that in the formation of enamel of

FIG. 14. Enamel forming organ of rat. Tissues teased apart to show different phases or elements of enamel organ. *A*, Loops of blood vessels pulled out from the secreting papillae as seen at *B*; *C*, ameloblasts; *D*, enamel; *E*, dentine.

certain fishes after the first thin layer of enamel is deposited, the ameloblasts disappear, *but the formation of enamel goes on.* Now it is highly improbable that the secretion of the calcific material for

FIG. 15. The enamel secreting organ of the rat magnified about 1,000 diameters. *A*, fully formed secreting papillae; *C*, nucleus of ameloblast; *D*, fibrous cytoplasm projecting from end of ameloblast.

enamel building is shifted about from one set of cells to another in different animals.

You begin to see now that my question, "What is the enamel organ?" has a very real significance. We may have the highest type

of enamel without a stellate reticulum. We may have a form of enamel even without ameloblasts, but no one, so far as I am aware, has ever shown that enamel can be formed in the absence of those cells which are developed from the inner or outer epithelial layers or from

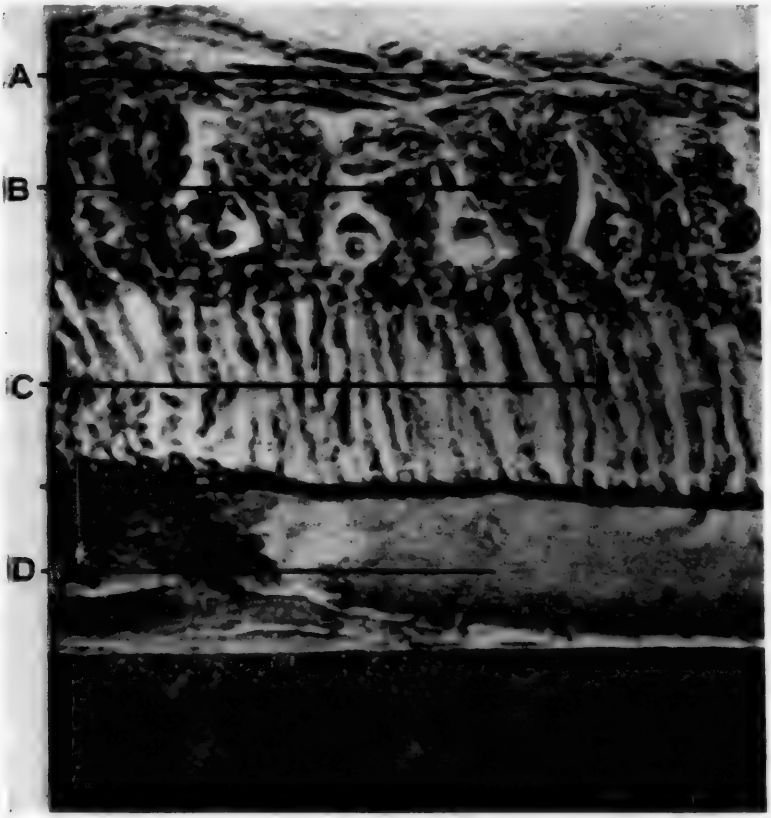


FIG. 16. Another view of the highly developed secreting organ of the rat. *A*, blood-vessel entering the group of papillae seen at *B; C*, ameloblasts; *D*, enamel.

both. These cells, then, constitute the most essential feature of the enamel organ. There is a great mass of evidence going to show that the function of the ameloblasts is chiefly that of giving form and structure to the *organic stroma* of the enamel rods, but there is no evidence,

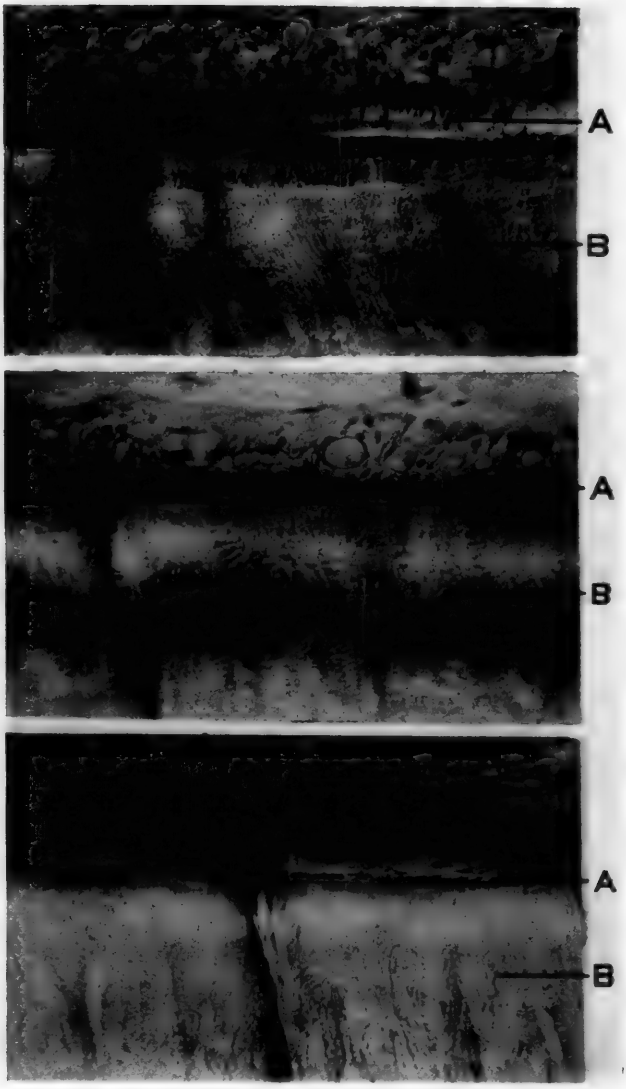
so far as I am aware, to show that they play any part in the secretion of the calcific material from which enamel is formed.[3] The ameloblasts lay down the organic matrix into which the secreting cells pour the calcific material. In other words, enamel, like dentine, has an organic matrix. In the completely formed enamel rods this organic matrix, supplied by the ameloblasts, can often be revealed by proper treatment. In my text and in the illustrations shown I have presented a considerable amount of data in the shape of demonstrated facts. My conclusions rest on this data. The only way to overthrow my conclusions is to destroy the data presented. Up to the present no one has ever made the attempt to do this. (Study *figs. 10* to *16*, inclusive.)

THE FORMATION OF NASMYTH'S MEMBRANE

The last act, so to say, in the development of enamel is the formation of Nasmyth's membrane. Hopewell Smith, Mummery, Paul and others have devoted considerable time to the investigation of this structure. They have shown that it is composed of two layers, an inner and apparently structureless layer, and an outer layer of calcified or cornified cells. But no one has given a convincing demonstration of just what cells are calcified to form this structure. I suggest that the inner, structureless part of this tissue is formed from that discrete layer seen between the inner ends of the ameloblasts and the forming enamel. Many years ago I gave this structure the name "inner ameloblastic membrane" and it is probably identical with Huxley's "membrana praeformativa." The formation of Nasmyth's membrane is most clearly seen in the persistently growing teeth of the rodents.

If we cut, by the Weil process, a very thin section of the lower incisor of a rat or a squirrel, we find that the ameloblasts are of about the same

[3] Dr. William G. Skillen in a very interesting paper on "the development and structure of the tooth apex," in the *Journal of the National Dental Association*, for January, 1921, speaking of the cells of the stratum intermedium, presents strong confirmation of my view of the function of these cells from another standpoint. He says: "Leon Williams has associated the cells of the stratum intermedium with enamel formation, and it is possibly due to the failure of these cells to descend as part of the stimulative layer, as well as the lack of continuity of the blood vessels, that no enamel is formed on the root." I entirely concur in this view.



FIGS. 17, 18 and 19. Demonstrate method of formation of Nasmyth's membrane in lower incisor of rabbit. The initials refer to the corresponding parts in the three views. Nasmyth's membrane is the transformed ameloblasts.

Fig. 17 is from near the middle part of the tooth and shows *A*, ameloblasts about the average size; *B*, enamel. *Fig. 18* was photographed from the same tooth much nearer the point where it emerged from the jaw. The ameloblasts at *A* are seen to be much smaller and to have assumed a semi-reclining position. *Fig. 19* from the same tooth just before it emerges from the jaw. Secreting organ and ameloblasts have disappeared. The ameloblasts have become converted into a layer of flat, nucleated cells which is Nasmyth's membrane.

length for the inner or formative half of the tooth. From a position about midway in the length of the tooth to the point of its eruption from the jaw the ameloblasts gradually decrease in length and also change their position relative to the surface of the enamel. The normal position of the ameloblasts is very nearly at a right angle to the long axis of the tooth. In the outer half of the tooth they assume a reclining position which increases until, just before the tooth emerges from the jaw, they are seen to lie perfectly flat upon the surface of the enamel. Up to the very point of eruption the nuclei of the cells take the stain perfectly so that each individual cell, and also the relation of the cells, is clearly seen. In some of the rodents the ameloblasts do not assume this reclining position but become increasingly shorter until they finally flatten out into a layer of but a single cell in thickness. Nasmyth's membrane, in the teeth of the smaller rodents, is very thin, not more than five or six microns in thickness.

Another very interesting feature in this connection, and one strongly confirmatory of the views I have presented concerning the functions of the various parts of the enamel organ, is the fact that the papillary layer maintains its form and apparently its function very nearly to the place where the tooth emerges from the jaw, where the remains of the ameloblasts lie thin and flat as the calcified outer part of Nasmyth's membrane. Just consider for a moment the significance of this. The papillary layer secretes the calcific material from the blood. It is, therefore, absolutely necessary that this, the most essential part of the enamel organ, should continue its functional activities until the remains of the ameloblasts are calcified into the outer part of Nasmyth's membrane. And this, we see, is exactly what happens. This rounds out and completes a theory of enamel formation in which there are no inconsistencies, a theory which is supported by all the known facts. (Study *figs. 17, 18* and *19* in their order.)

ARE ENAMEL RODS OF THE SAME DIAMETER THROUGHOUT AS CLAIMED BY NOYES OR DO THEY DIVIDE AND BRANCH AS DESCRIBED BY MUMMERY?

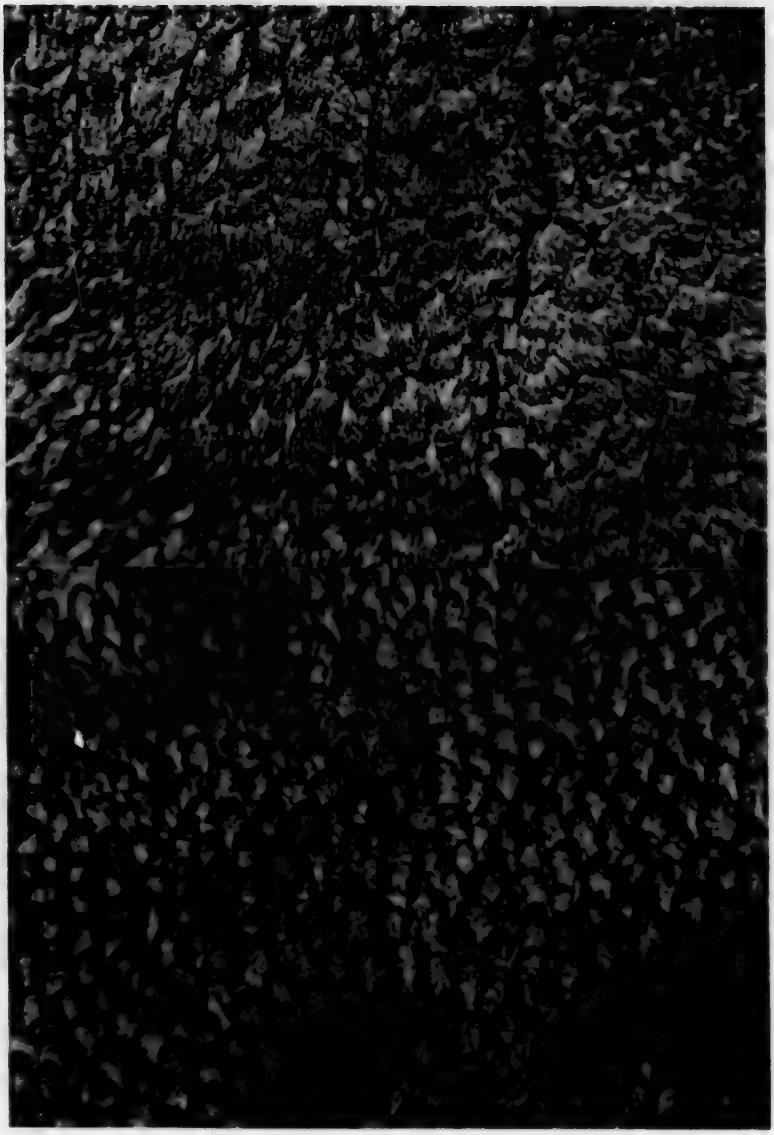
The fact that the outer surface of most teeth has an area of enamel nearly twice as great as it is at the dento-enamel junction makes

necessary an explantation as to how this increase in area comes about.

Noyes has stated, very emphatically, that the rods are of the same diameter throughout. Mummery, in his "Microscopic Anatomy of

FIG. 20. Shows apparent branching of enamel rods in fossil tooth of mastodon, at *A*. But this appearance is an illusion due to the spiral arrangement of the enamel rods. A spiral group or whorl of rods penetrates rods passing in another direction This gives the appearance of branching.

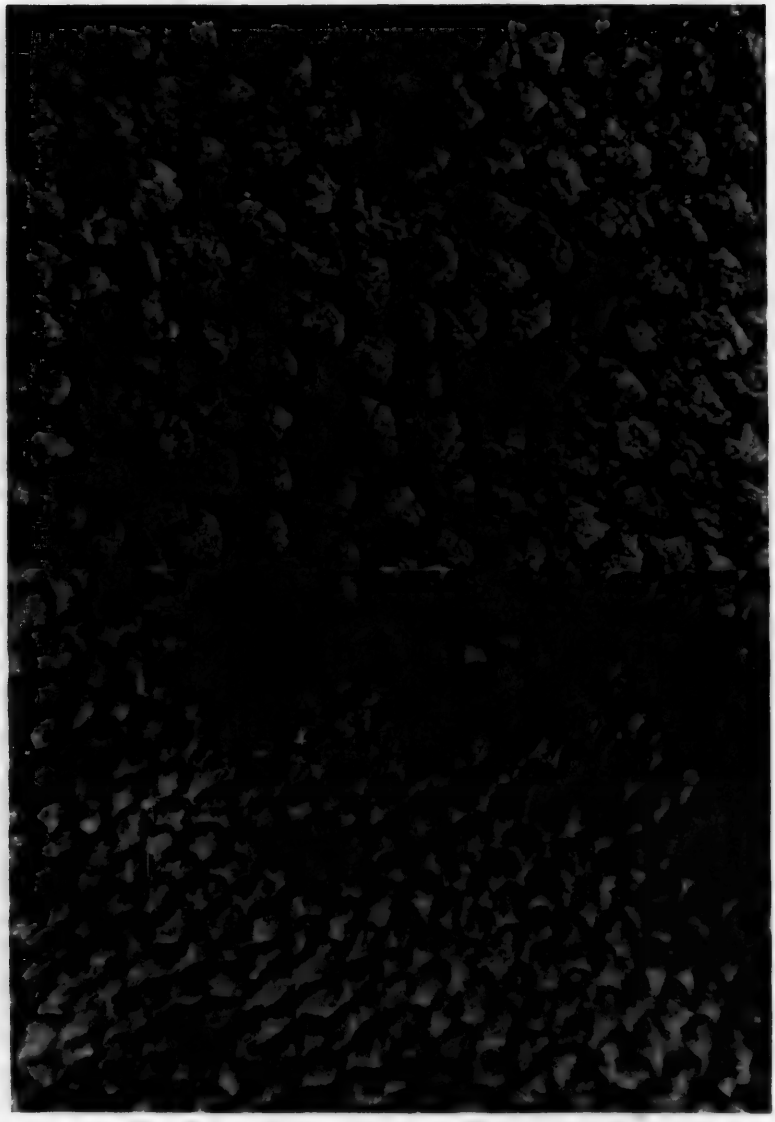
A



B

FIG. 21. Cross section of enamel rods from human molar, magnified about 1,200 diameters. *A*, near the outer surface of enamel; *B*, near the dento-enamel junction. This, and the following illustration, show conclusively that the enamel rods gradually increase in size from the dentine to the surface. There is, therefore, no need for the theory of branching enamel rods to explain the larger surface area of enamel.

A



B

FIG. 22. Cross sections of enamel from a human molar tooth; same degree of magnification. *A*, near the outer surface of the tooth; *B*, near the dento-enamel junction. This shows that the diameter of the enamel rods near the surface is nearly twice as great as at the dento-enamel junction. The theory that the larger surface area is provided for by branching of the rods is thus shown to be incorrect.

the Teeth," presents a photograph of the enamel of *Phacochoerus* (the wart hog), claiming that the photograph shows branching of the rods as they pass from the dento-enamel junction toward the surface of the tooth. I think this is a mistake of interpretation on Mummery's part. The study of a great many sections of many kinds of teeth has convinced me that this appearance of branching, which may be seen in nearly all forms of enamel, is always due to the tortuous

FIG. 23. Cross section of enamel rods from human molar. Magnified about 1,200 diameters. Shows many enamel rods have a much larger diameter in one direction than in the other. This is a condition frequently seen and would appear to be a necessity of the form and arrangement of the enamel cap.

courses and the consequent inweaving and outweaving of the rods among themselves. An enamel rod will often pass between the other rods and so give the appearance of branching. And when we come to examine Mr. Mummery's photograph critically we find that there are nearly or quite as many enamel rods which branch *toward the dentine* as toward the surface. This is very strong confirmatory evidence of

the explanation which I have suggested. I shall present additional proof of this view under another heading. Noyes is mistaken in his claim that the enamel rods have the same diameter throughout. He might occasionally find one of which this would be true but I have found that the ratio of diameter between dento-enamel border and surface is, in a general average, as 5.5 to 10.

Pickerill has also investigated this subject and the ratio measurements which he gives do not differ very materially from mine. I imagine that Noyes's mistake came about through the measurement of the rods in longitudinal section in which the differences, in most positions, is not great. But in transverse sections the difference in diameter of the rods is evident at a glance. But it should be observed that in nearly all instances there is a greater and a lesser diameter of the rods at the surface of the enamel, whereas at the dento-enamel junction the diameter is about the same in all directions. The form and structural arrangement of the enamel cap makes this fact necessary. (See *figs. 20, 21, 22* and *23*.)

THE LINES OF RETZIUS AND THE SCHREGER BANDS

A great deal has been written on these two subjects and ingenious and complicated theories have been offered in explanation of the appearances presented. The correct solution of the problem has been suggested by Pickerill and some others, but as in several other problems in dental histology the illustrative proof offered has never been adequate and convincing. The causes of these phenomena are really very simple when clearly understood. One of the most wonderful things in nature is the rhythmic movements in the growth and life of organic forms. One of the most fascinating books in the whole realm of science is Prof. D'Arcy Thompson's book on this subject.

The Retzius lines in their finest forms, as shown in fortunate sections of enamel, represent the rhythmic action of the enamel organ in the deposit of the globular sections of which the enamel rods are built up. When this functioning action of the enamel organ is perfect, a row of these globular bodies is deposited, simultaneously, around the entire circumference of the developing tooth. This row of globular bodies marks a developmental line completely around the tooth when observed in transverse section. When seen in longitudinal section

FIG. 24. The persistently growing lower incisor of the albino rat. The persistently growing teeth of the rodents supply the finest material for studying the developmental conditions and tissue relations of these organs. In some of the rodents, as, for instance, the rabbit and the beaver, both incisors and molars are rootless, persistently growing teeth. In others, as shown in this illustration from the rat, the incisor is a persistently growing tooth while the molar has a root and is permanently formed at the outset. A rather curious thing about the persistently growing teeth of the rodents which, so far as I know, has not been previously noted, is that the enamel stroma is laid down the full thickness almost from the commencement. Calcification then proceeds gradually from the end deep in the jaw to the point where the tooth emerges from the jaw. And it is almost certain that the hardening process of enamel continues in all teeth for some time. In permanently formed teeth perhaps for years after complete eruption.

the lines run obliquely from the dento-enamel border to the surface. During the course of the development of a tooth, especially among the so-called highly civilized races, there are many fluctuations of all the vital activities, particularly fluctuations in the nutritive functions.

FIG. 25. Section through the jaw of young rabbit, showing persistently growing cheek teeth. These teeth are covered with enamel throughout their entire length and there are complicated infoldings of enamel through the body of the tooth. Enamel is constantly being formed on all that part of the tooth which is in the jaw and there is nowhere any trace of a stellate reticulum. The manner in which the enamel of these teeth is formed is shown in figure 12.

Marked fluctuations of this character seem to have a double effect associated with the production of the Retzius lines. There is some molecular disturbance in the forming enamel which changes the action of the light waves in both reflected and transmitted light, and

FIG. 26. Section of molar tooth of beaver. The sectional infoldings of enamel are plainly shown in this illustration. At the plane of the section shown there are six enamel bands running the entire length of the tooth. The external surface of the tooth is also covered with a thin layer of cement.

FIG. 27. The left lower half of the mandible of the beaver. The inner wall of the jaw has been removed to show the great length of the incisor tooth and the manner in which it is placed in the jaw.

there is also, in some instances, slight pigmentation of a number of these rhythmic deposits, thus giving the effect of a colored band of

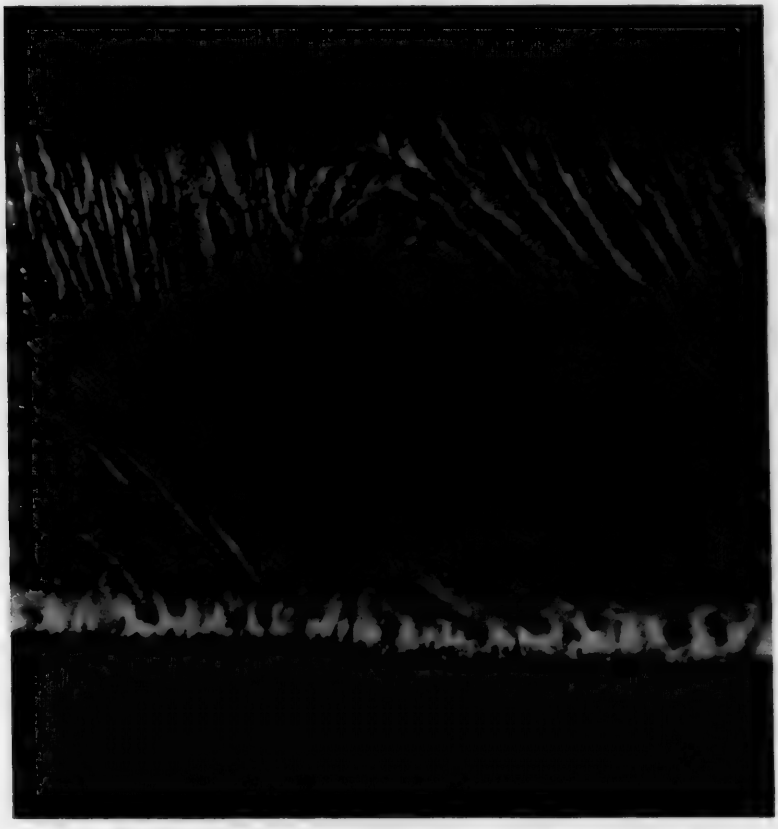


FIG. 28. Section of enamel from incisor of Belgian hare. A certain modification of the spiral arrangements of the enamel rods is beautifully shown here. The contention that the spiral arrangements of the rods in human enamel represents abnormal or imperfect formation is seen to have no foundation in fact. This spiral arrangement is the most characteristic feature in nearly all forms of enamel and adds greatly to its power of resistance to pressure.

greater or less width. But for the most part I think the appearances of color are due to varying molecular conditions which affect the transmission and reflection of light. But, as I have said, the appear-

ance of the lines themselves is due entirely to the rhythmic action of the enamel organ. The simultaneous deposit of a row of enamel globules or segments of rods around the entire circumference of a tooth produces the effect of a line, the Retzius line. When several of the rhythmic deposits are produced under the influence of a nutritive or other functional change we see a band of greater or less width, but all of these bands are really composed of the lines I have described.

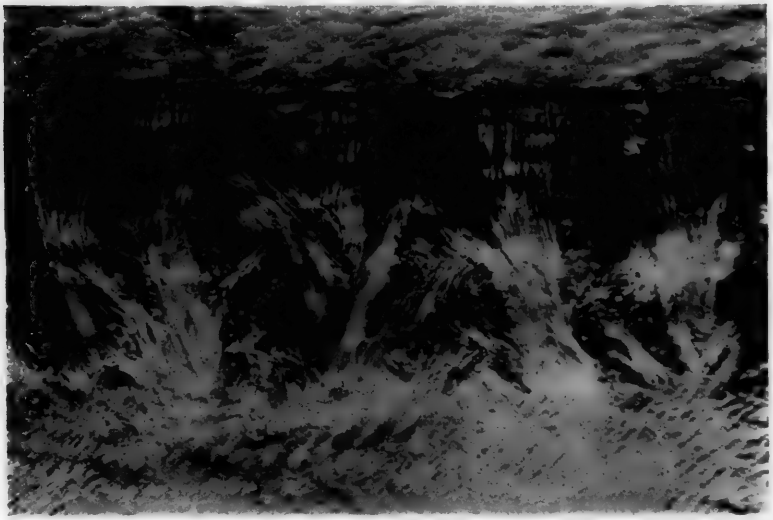


FIG. 29. Enamel, in longitudinal section, from the molar tooth of the Belgian hare. This is the most striking modification of the spiral arrangement of enamel rods that I have found.

The Schreger bands also are a purely structural phenomenon and present one modification of a fundamental form arrangement of the rods in all varieties of enamel. That fundamental form arrangement is the spiral. The arrangements of the rods in all forms of enamel are varied modifications of the spiral, the Schreger bands, perhaps, approaching most closely to the ideal form.

To all who are familiar with only the conventionally cut sections of enamel, mostly straight longitudinal sections, the real structure of this tissue, especially in the rodents, as revealed by sections cut at a

variety of angles, will, I imagine, be something in the nature of a surprise.

FIG. 30. Transverse oblique section from incisor tooth of red squirrel. What appears to be a decussating arrangement of the enamel rods, when viewed in the plane of two dimensions, is seen to be a spiral arrangement when considered in terms of three dimensions in which the rods actually exist.

Take, for example, the enamel of the beaver. What Tomes and Mummery have described as the decussating layers in the inner part of this enamel, alternate layers passing in opposite directions, are, in

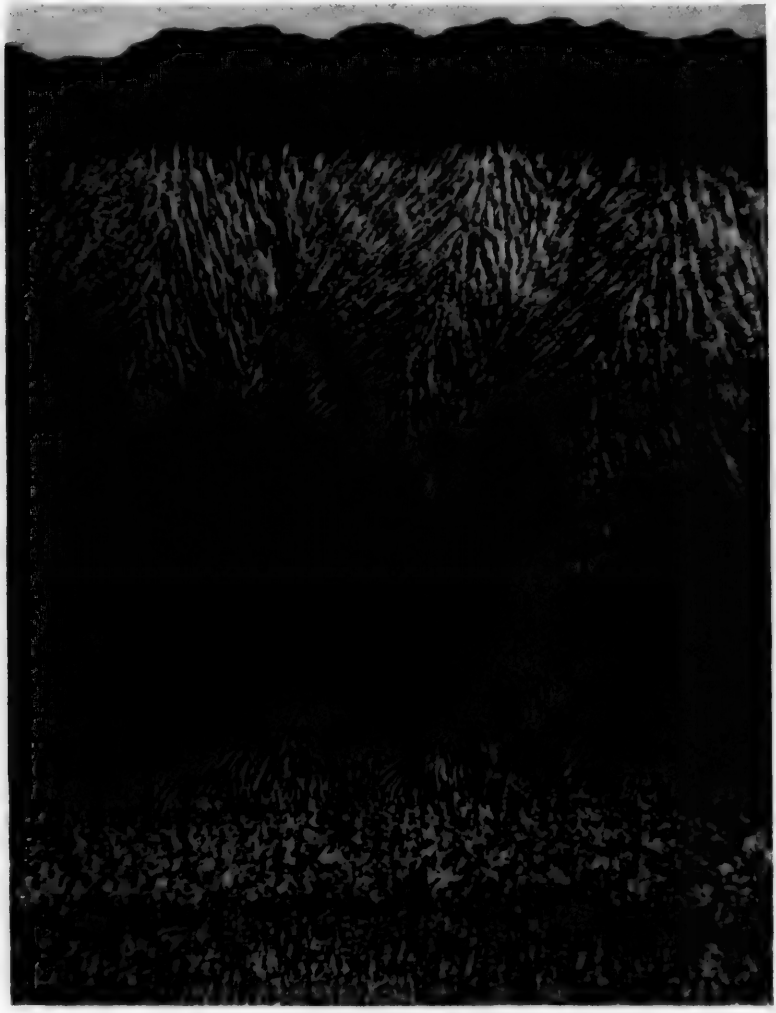


FIG. 31. Section of enamel of porcupine. The spiral arrangement of the rods is beautifully shown in this specimen.

reality, as we shall see, a wonderfully complicated modification of spiral form arrangements. We shall see bundles of a dozen or a score of enamel rods twisted into the forms of ropes and these rope-like

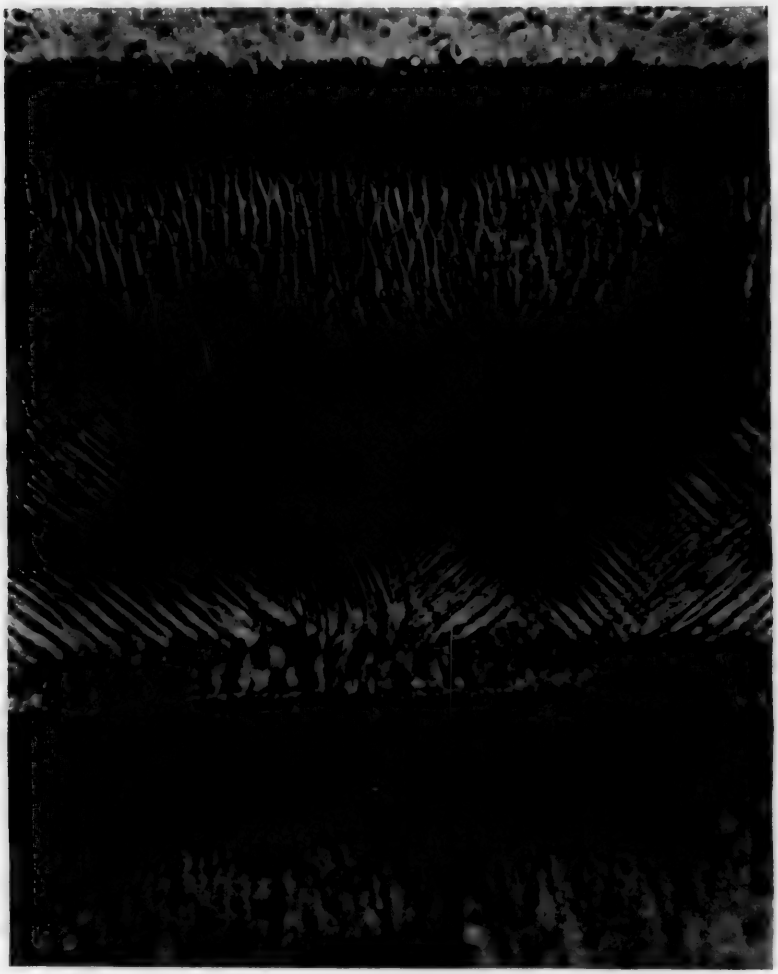


FIG. 32. Oblique section of enamel from lower incisor of muskrat. Another modification of the spiral is seen in the course of the rods in the inner portion of the enamel. In the outer part the rods are cut almost transversely.

bundles arranged into forms resembling woven fabrics. We shall see how perfectly adapted these complicated form arrangements of the enamel rods are to meeting the great stress requirements to which the teeth of the rodents are subjected. It is a beautiful illustration of

FIG. 33. Enamel of porcupine showing that the enamel rods are continuous with the ameloblasts. The ameloblasts are shown at *A* and the enamel rods at *B*. When we remember that the ameloblasts are always very regularly arranged and parallel to each other in all forms of enamel, it seems difficult to form any conception of the movements and play of forces which result in the very complicated arrangements of the rods in many forms of enamel.

the working, through a million or two millions of years, of the principles of evolution and adaptation. And running through all the varied form arrangements of the enamel rods, in all animals, we shall see more or less constantly in evidence modifications of that arrangement which in human teeth is known as the Schreger bands.

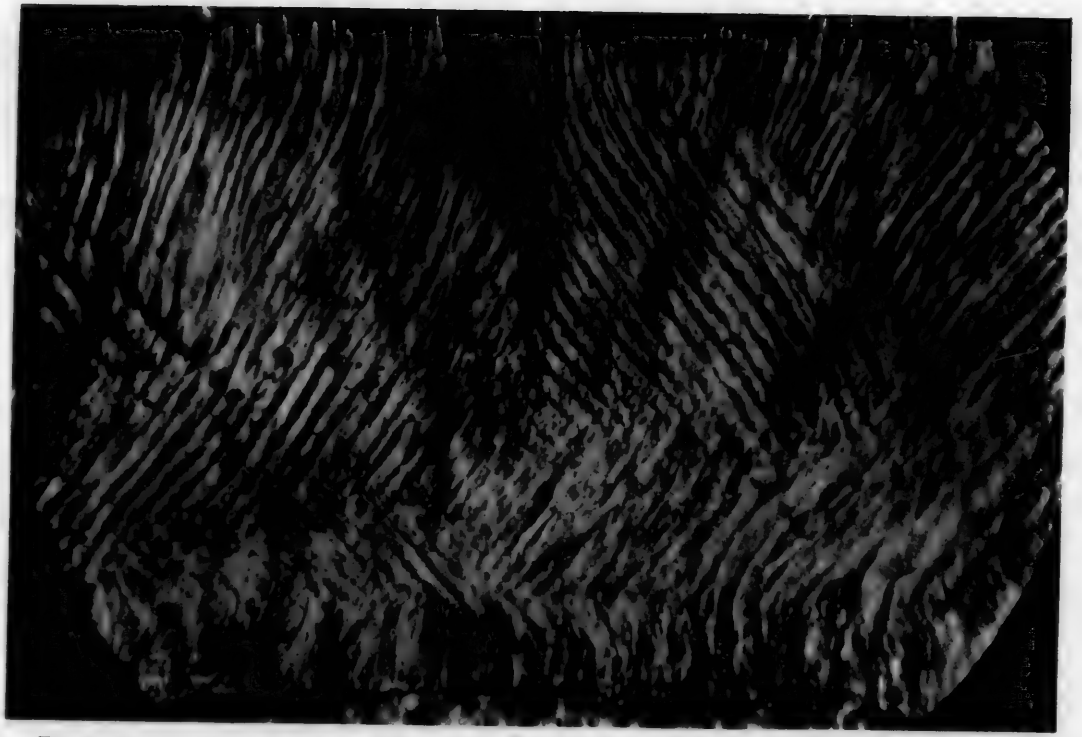


FIG. 34. One aspect of the arrangement of the enamel rods in the incisor of the beaver. The rod arrangement in the enamel of this animal is the most complex of any that I have studied. It is this complex structure that gives the great strength necessary to the use which the beaver makes of his incisor teeth.

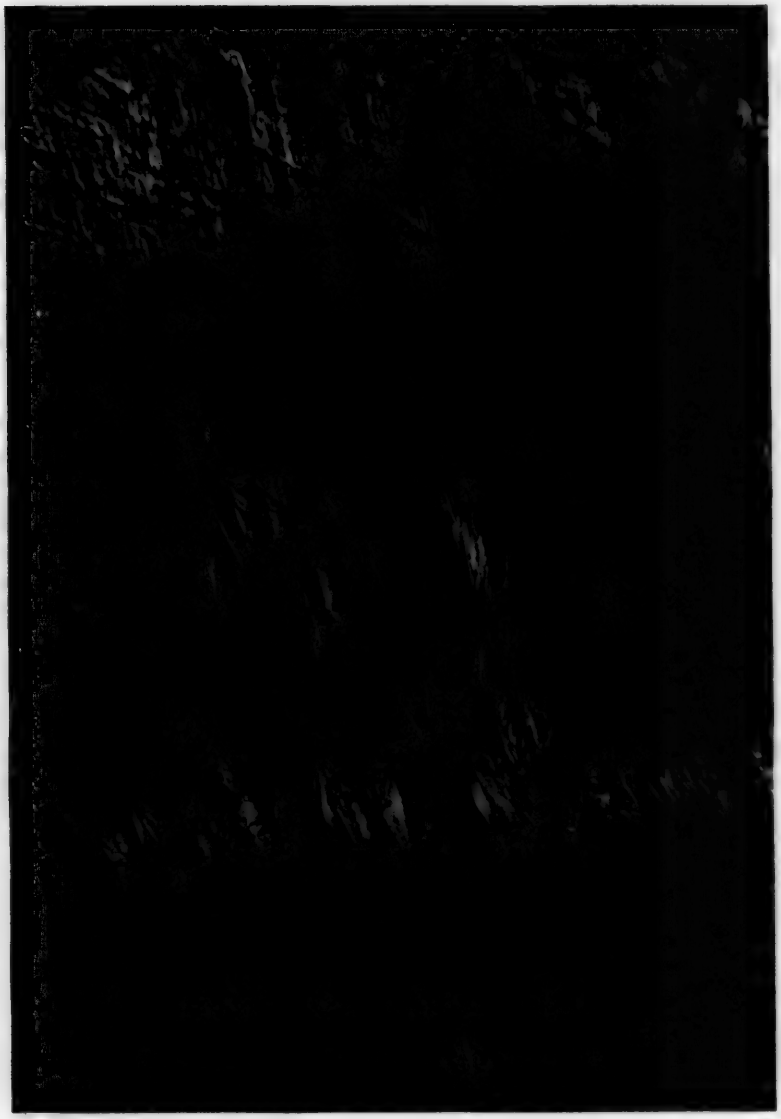


FIG. 35. Longitudinal section of enamel of beaver. It is plainly seen that the enamel rods have a fibrous stroma into which the calcific material is deposited.

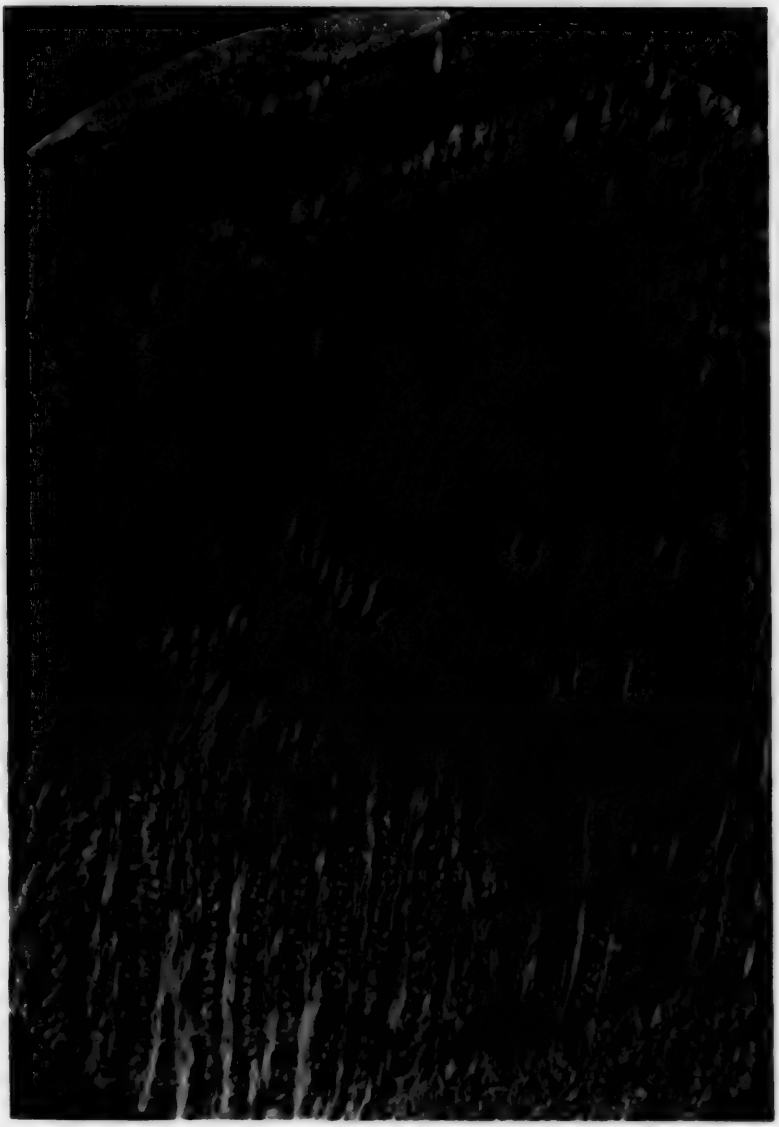


FIG. 36. From longitudinal section of lower incisor of beaver. The arrangement of the outer half of the enamel rods is seen to be totally different from previous representations. The enamel rods pass in several directions and are interwoven like the warp and woof of a fabric.

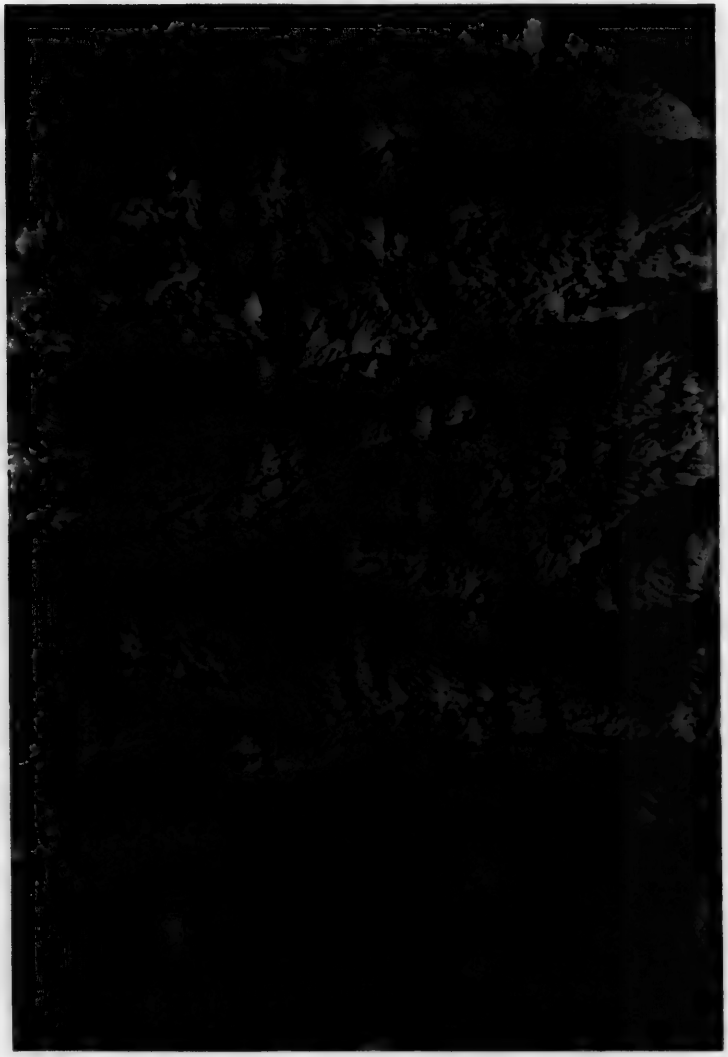


FIG. 37. Enamel rods from a section of the tooth of the beaver ground on a plane parallel to the surface of the tooth. The magnification in the view is about 200 diameters and what at first sight appear to be enamel rods are really bundles of twisted enamel rods. Each bundle contains a considerable number, probably ten to twelve, enamel rods. This arrangement is always seen near the surface of the enamel of the incisor teeth of the beaver. It is easily seen what enormous strength and resisting power this arrangement gives to the enamel of the beaver.

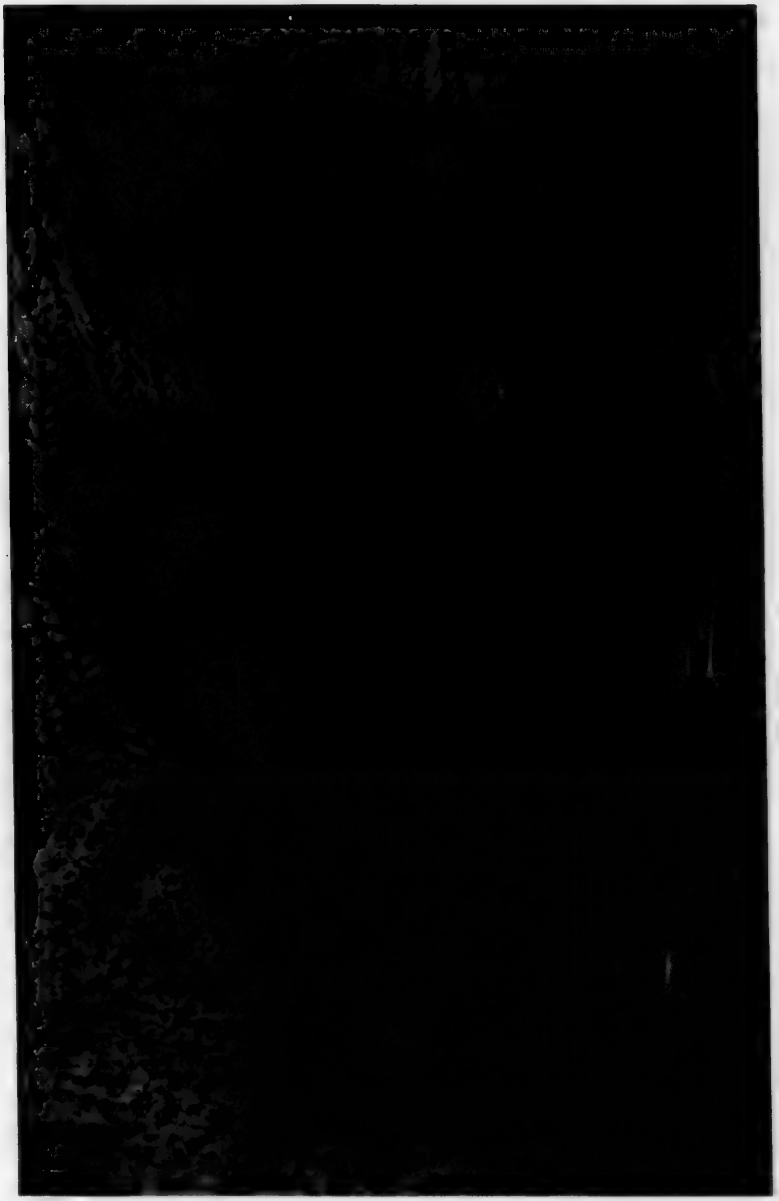


FIG. 38. Another view of the enamel of the beaver, magnified about 600 diameters, in which it is seen that in every alternate plane of the enamel rods the arrangement of the sections is in an opposite direction. This also gives greatly increased strength to the enamel.

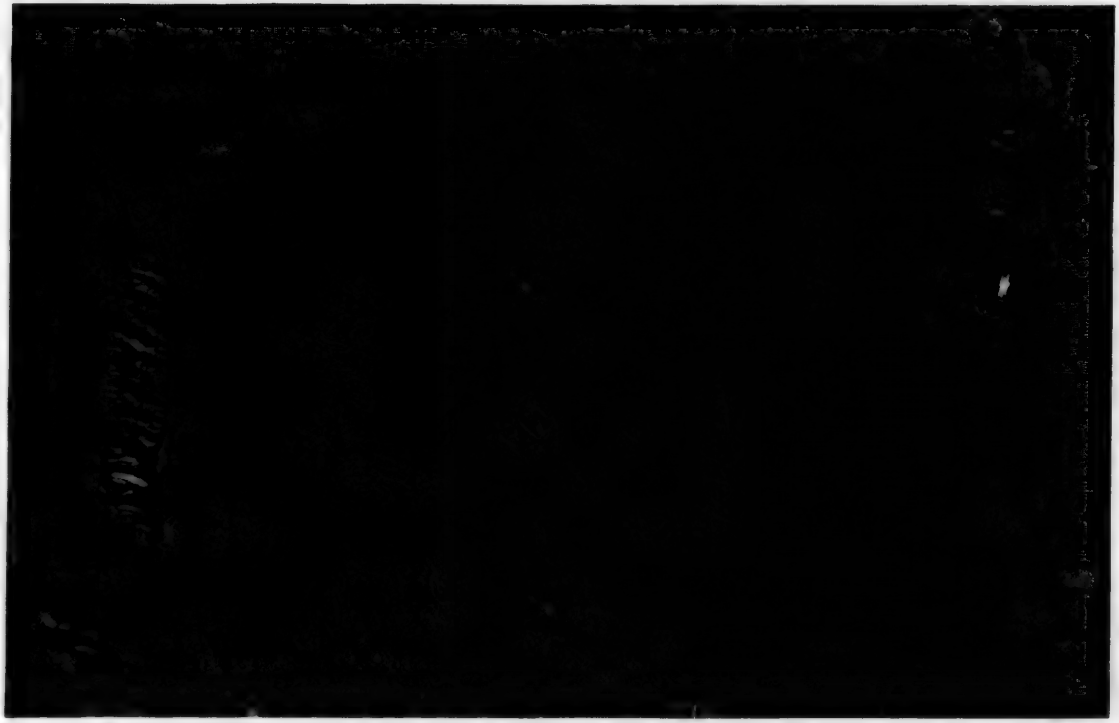


FIG. 39. Transverse section of enamel from the incisor of the woodchuck. The enamel rods are here seen in both longitudinal and transverse sections. The transverse sections of the rods plainly show, in sections, the plasm strings which constitute the fibrous stroma of the enamel rods. Magnified 1,000 diameters.

Here, as in many other departments of natural history, it seems as if we were catching glimpses of some great intelligence playing with a few fundamental structural and form ideas, making endless experiments of modification and adaptation. These structural arrangements in the rods in different forms of enamel should, I think, be

FIG. 40. Transverse section of lower human molar. Transverse sections of teeth are not often shown but such sections yield much new and valuable information, especially concerning the conditions of structure and relationship at the dento-enamel border.

classed among the very wonderful things of nature. When we remember that there is never any suggestion of these complex arrangements in the ameloblasts from which the rods are formed, it seems impossible for us to construct any mental picture of the orderly, rhythmic and exceedingly intricate movements necessary to produce these effects. (Study *figs. 24* to *48*, inclusive.)

FIG. 41. From transverse section of human molar. The so-called "enamel spindles," which are extrusions of organic matter from the dentine into the enamel, are here shown deeply stained with eosine. The spiral arrangement of the rods in human enamel is well shown in this illustration and in the following one.

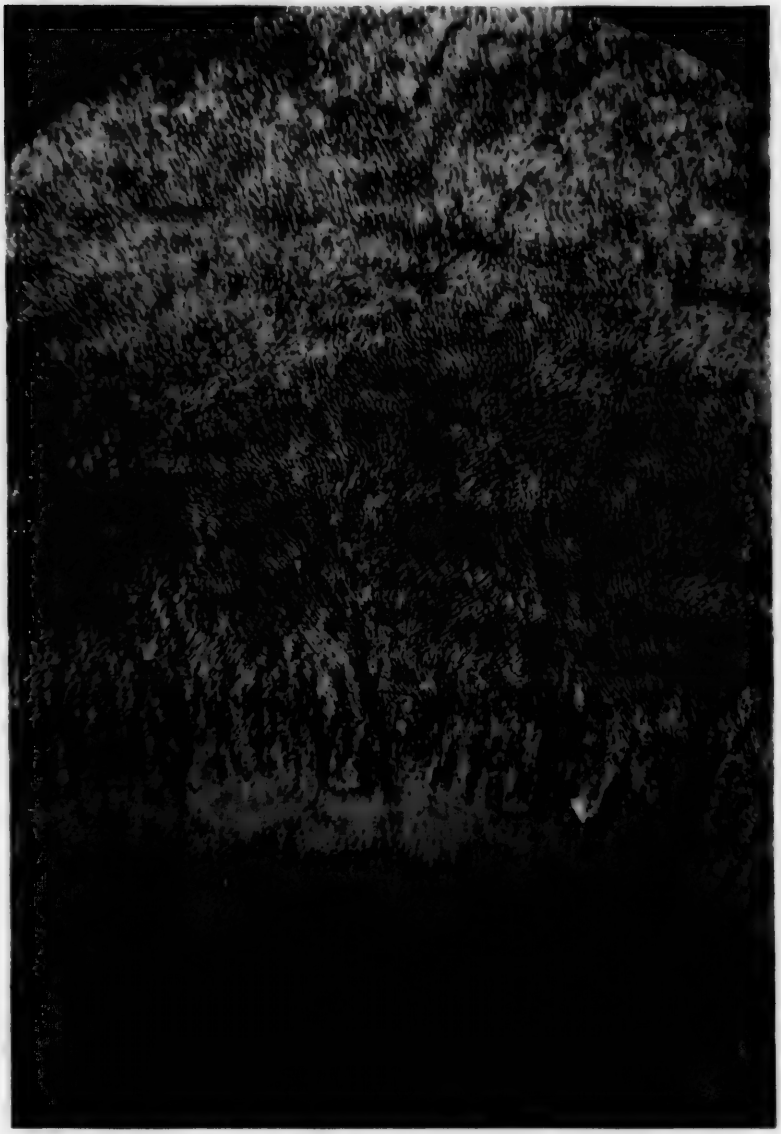


FIG. 42. Similar to fig. 41, but the spiral arrangement of the enamel rods is even more marked.

FIG. 43. This photograph, from a longitudinal section of an upper central human incisor, shows very clearly the true nature of the Retzius lines. They are seen to be produced by the rhythmic deposit of the globular bodies of which the rods are built up. A row of these globular bodies is delivered from the ameloblasts simultaneously around the entire circumference of the tooth. This gives the appearance of a line in longitudinal section. Slight differences in molecular structure, probably due to nutritional variations, when involving several lines of deposit, give the appearance of a band. The appearance of color is partly an optical effect and also partly due to pigmentation, at least in some instances.

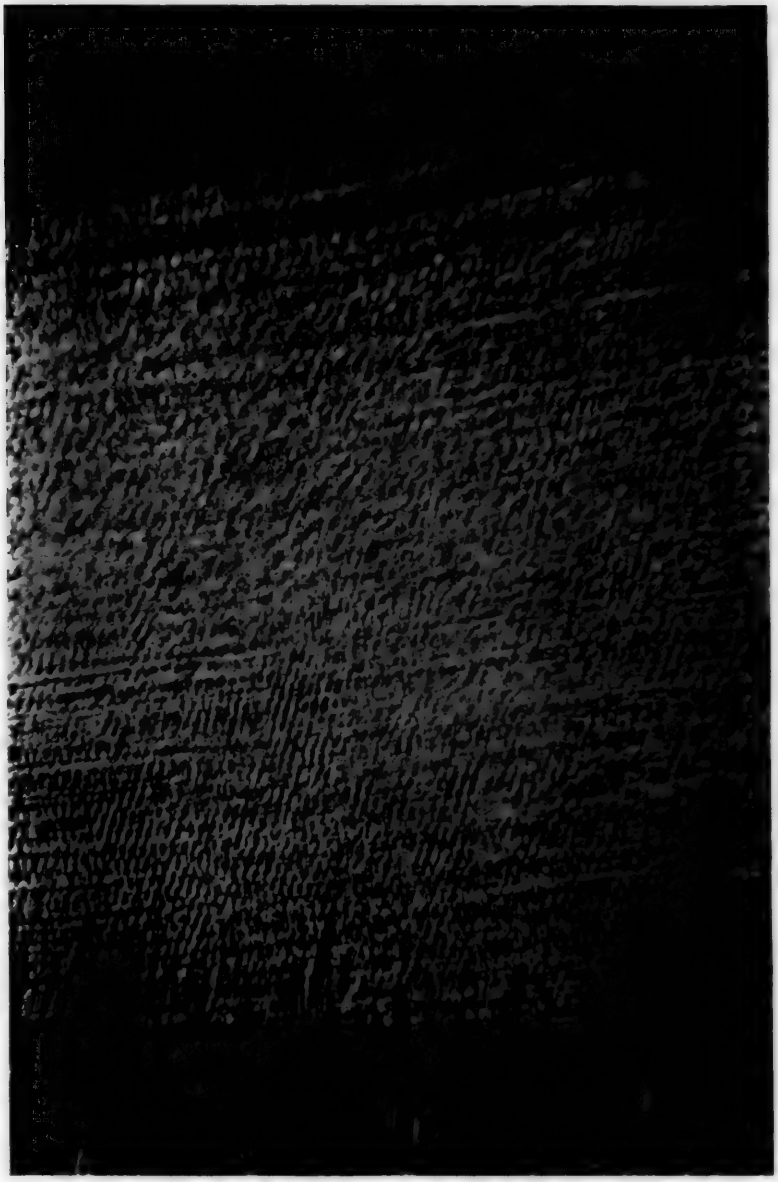


FIG. 44. Section from tooth of Tasmanian wolf showing deposit lines similar to figure 43.

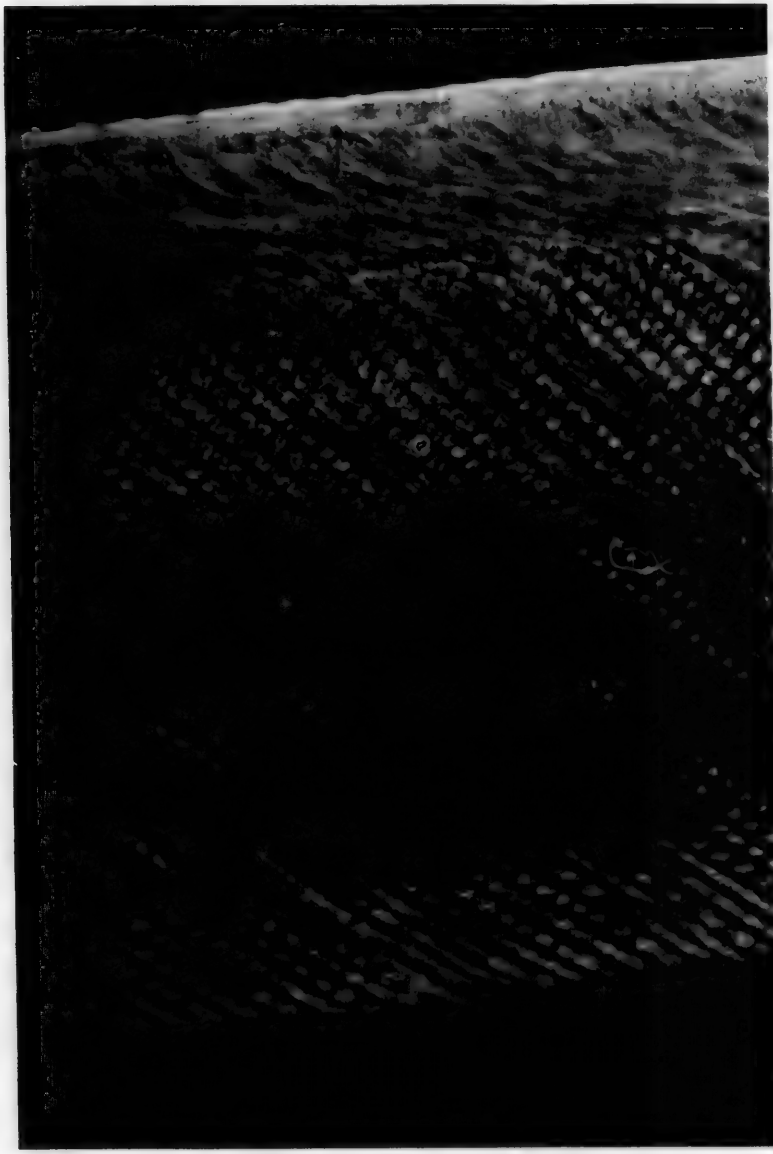


FIG. 45. From the incisor of the albino rat showing how the enamel rods are built up by the successive deposit of globular bodies. The fibrous stroma into which these globular bodies are deposited is also plainly shown in several places.

DEGENERATIVE CHANGES IN THE ENAMEL OF THE GUINEA PIG AS THE RESULT OF EXCLUDING VITAMINES FROM FOOD

It may seem to disturb a little the harmony of this paper to introduce the subject of pathology of enamel. But, as I stated at the outset I want to clear the ground as completely as possible for a future

FIG. 46. Longitudinal section of tip of lower human cuspid, showing the Schreger bands.

paper in which I shall consider the possibilities of chemical and metabolic changes in enamel, and if such changes occur, some of them will be of a pathological character. The photographs which I am showing to illustrate this section of my paper are from teeth sent me by Dr. Percy Howe of the Forsythe Institute, Boston. Most of you are no doubt familiar with the important work which Dr. Howe is doing. I

FIG. 47. Section from human tooth showing that the appearance of the so-called Schreger bands is entirely due to the spiral form-arrangement of the enamel rods, which is very finely exhibited in this illustration.

believe his experiments have been chiefly concerned with the results of excluding vitamine C, the anti-scorbutic vitamine, from food. Dr. Howe in his experiments has relied mainly on the guinea pig which seems especially sensitive to the production of scurvy. As Dr. Howe

FIG. 48. Another aspect of change in direction of the enamel rods which produces the effect of the Schreger bands.

has, in several papers, reported such effects of excluding the water-soluble C vitamine from food as may be observed with the unaided eye, I shall not take up this aspect of the subject, but shall call your attention very briefly to some of the effects of this treatment on enamel formation as revealed by the microscope, and these effects I have

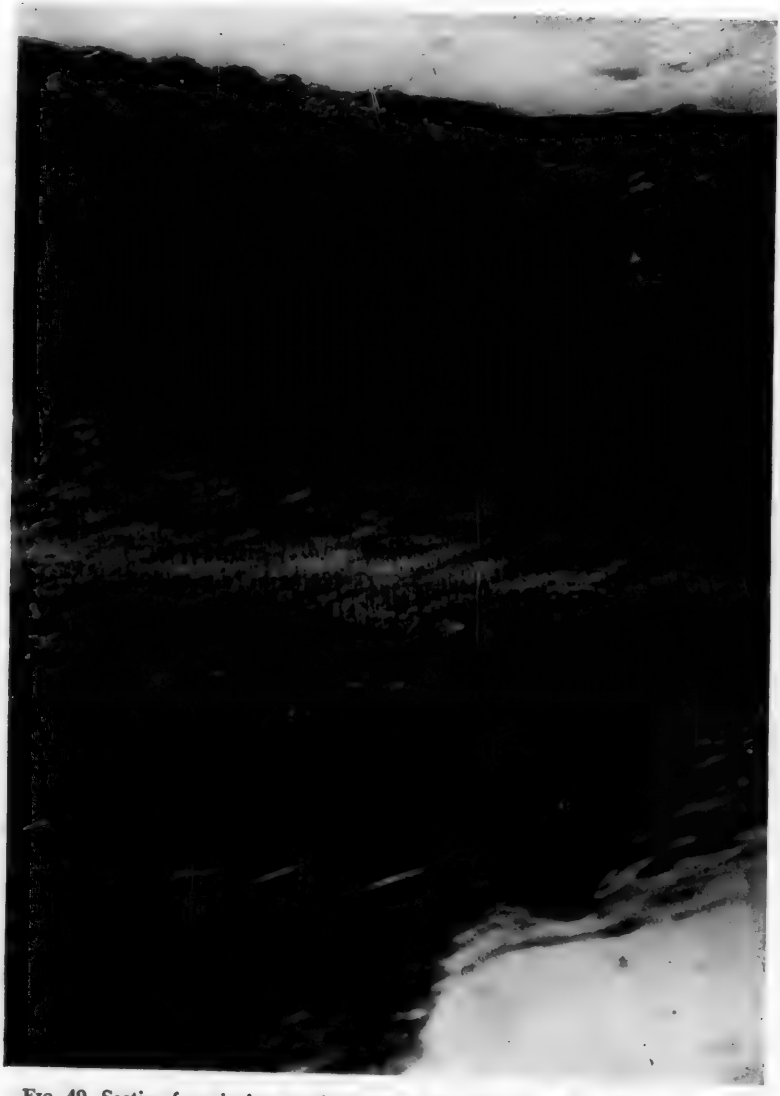


FIG. 49. Section from incisor tooth of guinea pig, from Dr. Percy Howe's collection, showing profound degenerative changes in enamel and dentine as the result of feeding vitamine-free food.

found surprising and astonishing. But although we are able to show that the exclusion of one or more of the vitamines from food produces profound effects upon the hard dental tissue, yet this is only a small

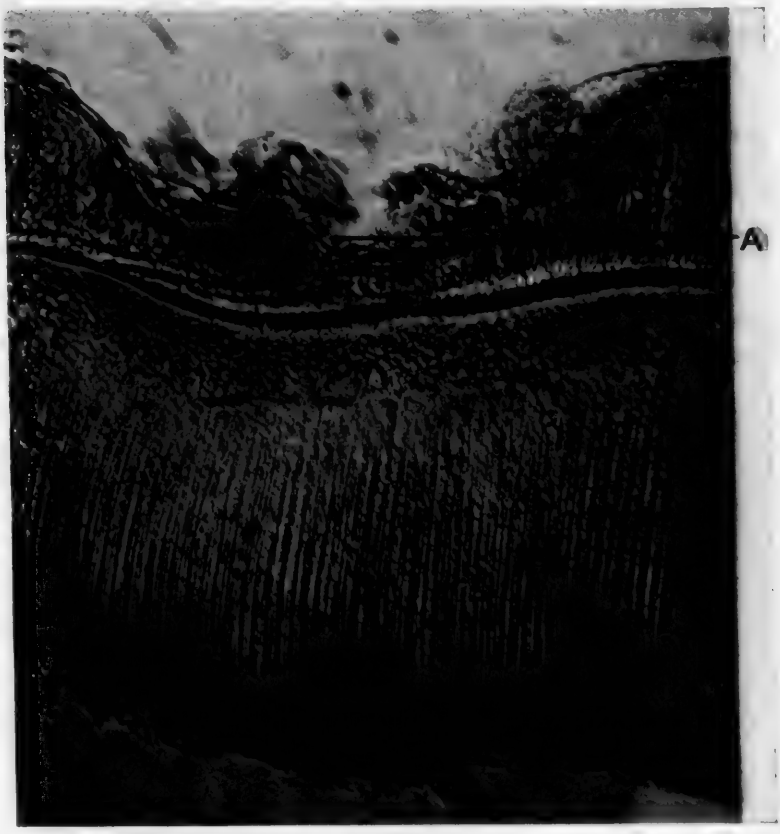


FIG. 50. From the same tooth as the foregoing. Shows complete breakdown of enamel formation at *A*. Compare this appearance with the next illustration which shows a similar failure of enamel formation in a syphilitic human tooth.

step toward the solution of our main problem which, as I have already stated, is that of determining whether there are chemical and metabolic changes in the enamel of fully erupted human teeth and under what conditions such changes occur if they take place at all.

Let me once more point out the great difference between experiments on the persistently growing teeth of the rodents and human teeth. The persistently growing teeth of the rodents (at least that part which is in the jaw) are always in the developmental stage. The enamel cells in the full-grown animal are still persistently active.

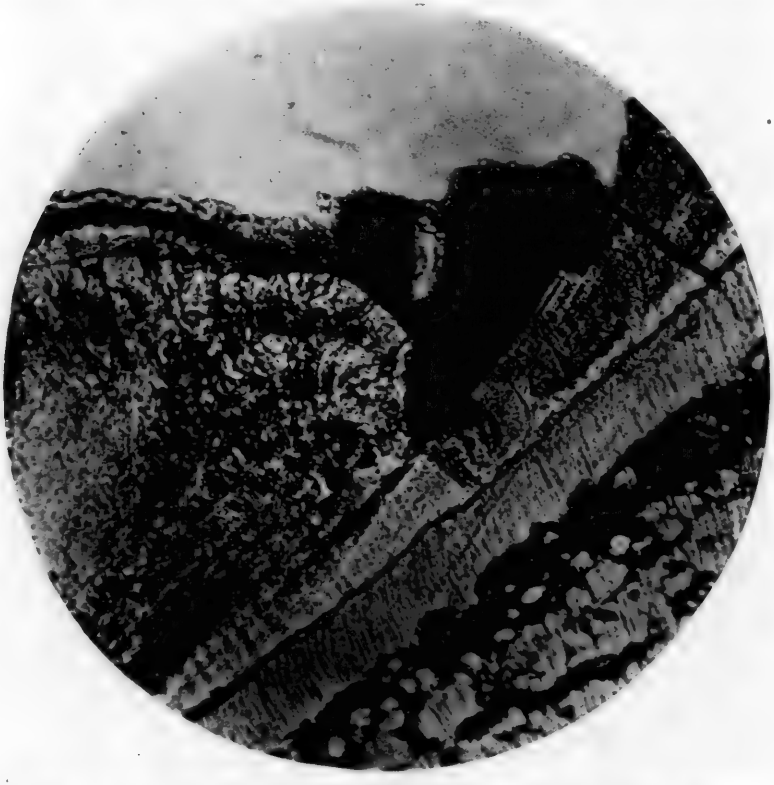


FIG. 51. Section from syphilitic human tooth, showing failure of enamel formation.

They are therefore ready to record in the forming enamel any deficiencies in diet. But the condition with fully erupted human teeth is very different. The enamel organ through which nutritive material for the formation of enamel is supplied has disappeared. This fact makes it necessary to completely change the statement of our prob-

lem. The question we now have to ask is, "Can metabolic changes take place in a tissue the formative cells of which have disappeared?"

There is one feature in these photo-micrographs to which I especially wish to call your attention with reference to what I have already said about the functions of different parts of the enamel organ. In congenital pathological or abnormal conditions of enamel there are two very distinct conditions. One of these conditions seems to pertain especially to the ameloblasts. In the hereditary effects of syphilis, for example, we find that the ameloblasts are often injured or destroyed and as a consequence there is no normal rod formation in the enamel. The ameloblasts, as I have pointed out, lay down the organic stroma which gives form to the enamel rod. When, therefore, they are injured or destroyed this organic stroma is not formed and we get an amorphous mass of more or less calcified material in place of the rods or, sometimes, a complete absence of enamel.

In the other condition we may have rod formation nearly or quite perfect but a defective formation of cement substance. This is the condition in the congenital white spots sometimes seen in enamel. The whiteness is an optical effect due to absence of the cement substance. This was the condition which Drs. Black and McCoy found in their studies of mottled enamel. This pathological condition seems to be the result of lack of functioning of the secretory part of the enamel organ. We have here, then, a confirmation of the view which I published in 1906 that the enamel organ has two distinct functions, that of the ameloblasts, which are the rod formers, and the secreting portion proper of the organ which supplies the calcific material. Both of these functions seem to have been profoundly disturbed in the teeth of Dr. Howe's guinea pigs. (See *figs. 49*, *50* and *51*.)

NEW PROOF OF THE PART WHICH THE BACTERIAL PLAQUE PLAYS IN DECAYING ENAMEL

On January 12, 1897, almost exactly twenty-five years ago, I read a paper before the New York Odontological Society on the pathology of enamel, in which I showed, for the first time, photo-micrographs of the bacterial plaque *in situ* on enamel. The evidence of changes taking place in enamel immediately beneath the bacterial plaque

was so clear that, at the time, there was no thought of challenging the view held as to the part which this film of microörganisms plays in caries of enamel.

Since that time Pickerill and others have expressed doubts about the bacterial plaque being the chief cause of enamel decay. This is the first time that I have ever replied to any of these criticisms and as Pickerill's statement sums up all the objections to which my attention has been directed, I will ask your indulgence for quoting that statement in full. He says:

FIG. 52. Section of lower human molar, showing, at *A*, early stage of caries of enamel.

It is contended by Leon Williams and Black that the essential feature in the pathology of caries is the presence of *bacterial plaques* upon the surface of the teeth, and that the lactic acid is secreted as such by the organisms themselves. Since this has a bearing upon the preventive treatment of caries, it is necessary to consider the importance of this claim.

1. The presence of plaques is undoubted, but they occur on all surfaces of teeth, and not solely on the mesial and distal surfaces, which are most liable to attacks of caries. They occur on the teeth of immune people apparently as frequently as upon the teeth of susceptible persons. They are found upon animals' teeth; they are found upon the inner surface of

secondary enamel decay. A bacterial film may be demonstrated, too, upon the surface of teeth in which the process of caries has been naturally

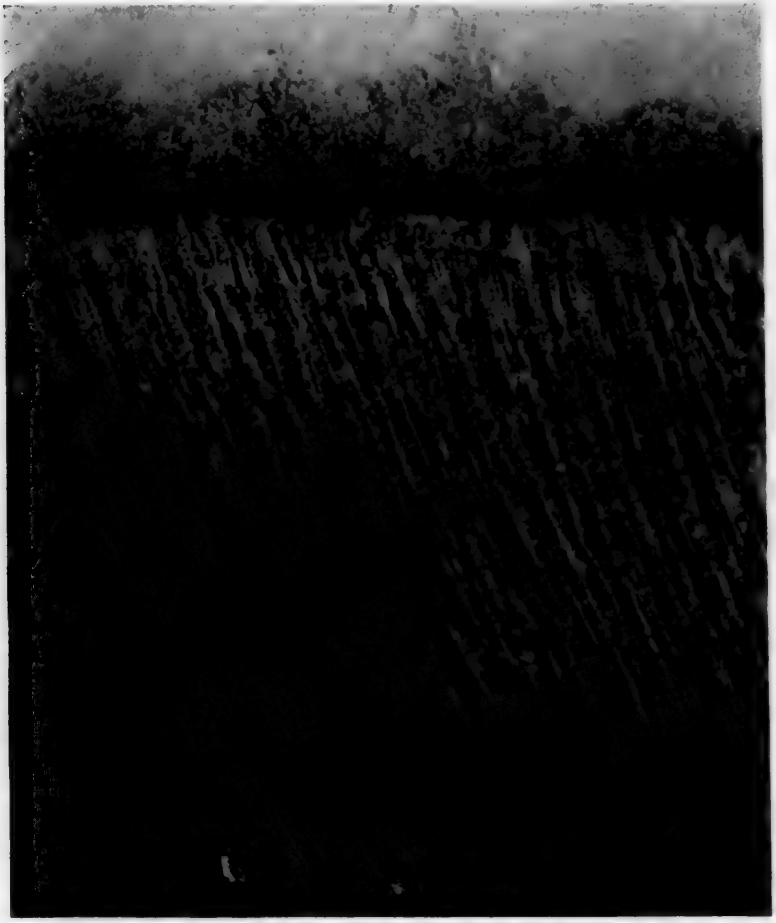


FIG. 53. Section from proximal surface of human tooth showing bacterial plaque *in situ*.

arrested and the dentine become quite hard, where, if anywhere, one would expect an *acid-forming* film of bacteria to be absent.

2. If the film of organisms is the essential origin of the acid, it would tend to constantly remove itself by dissolving its base of attachment.

3. The film of organisms cannot produce acid from Nasmyth's membrane nor from the oral secretions. They must have a supply of carbohydrate medium for the purpose and this must remain in apposition for a sufficient length of time for enough acid to be 'secreted.'

4. The saliva, which almost immediately permeates all food upon mastication, contains millions upon millions of organisms capable of transforming carbohydrates into acids. Why is it then necessary to look to any particular colonies for the formation of that acid?

We therefore come to the conclusion (1) that *bacterial plaques* upon the teeth are *not an essential factor* in the causation of caries; (2) that the lodgment and infection of carbohydrate material is the essential factor, and that those circumstances which favor the formation of bacterial plaques upon the teeth also favor the lodgment of carbohydrates, and so the occurrence of the two usually coincides.

Therefore it is evident that the two essential attacking forces in this disease are (1) the acid-forming organisms of the mouth, (2) the presence of fermentable carbohydrates—the first *active*, the second *passive*.

Immediately following this statement Pickerill points out that the phenomena of decay are, as he states it, "profoundly modified by a large variety of factors." And he proceeds to point out some of these factors. With all of this part of the views which he expresses I am in entire agreement. I also agree that the film is found on teeth which are not decaying and also in cases of arrested decay. But all of this is really outside the main question. It has to do with the problem of immunity and non-immunity, a subject about which we know but very little.

I have to confess to something of astonishment at finding Professor Pickerill's objection to the views held by the late Dr. Black and myself based on the fact that decay does not always occur beneath a bacterial plaque. Are not many forms of pathogenic bacteria which are known to be the active causes of specific diseases found in various parts of the bodies of individuals who have never suffered from those diseases? This is a familiar fact known to every bacteriologist. All that we know is that there is present in immune individuals some substance or some condition which inhibits the harmful action of pathogenic bacteria. And these conditions apply in caries of enamel as in other diseases. Furthermore, not all of the organisms found in the bacterial plaque are acid producers. Probably in all

instances the majority of them are not. In some instances there may be no acid-producing bacteria present. But all of these conditions do not touch the main issue, which is this: when acid-producing organisms are present in or beneath the bacterial plaque, the most favorable

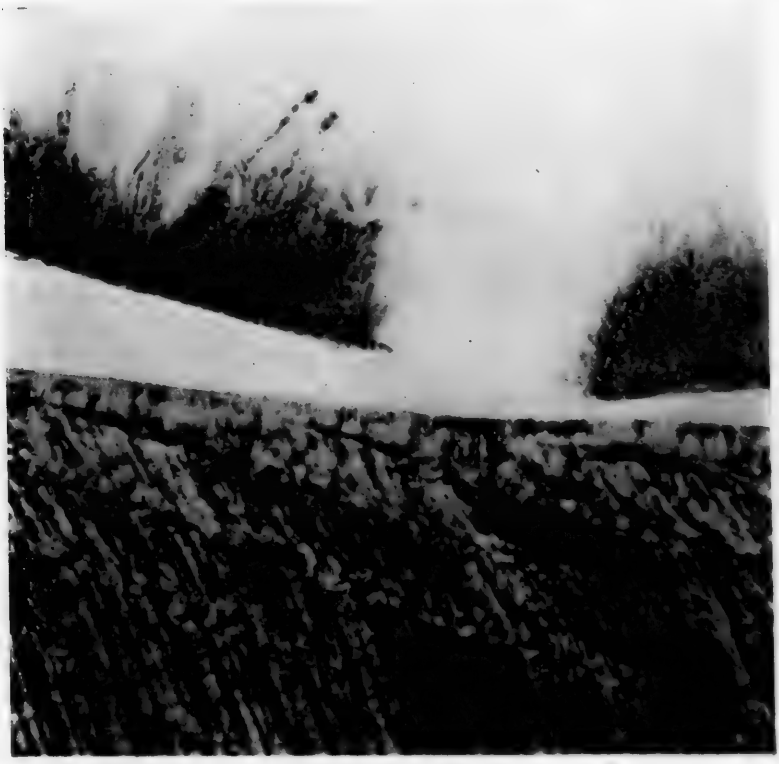


FIG. 54. Similar to figure 53 but showing the bacterial plaque raised by pressure on the cover glass.

condition is present for the decay of enamel. There is the gist of the whole matter.

The film acts as a sponge to absorb and hold the carbohydrate materials just where the acid-forming bacteria can produce the most destructive effects. The film protects the acid-forming bacteria in

their destructive work. All of this is so evident as to need no elaboration.

Professor Pickerill again astonishes me when he offers as one objection to my views the curious statement; "If the film of organisms is the essential origin of the acid, it would tend to constantly remove itself by dissolving its base of attachment." In making such a

FIG. 55. Looking down on the surface of enamel at *A*, from which the bacterial plaque has been removed, we see many openings. These openings are enlargements of natural canals, which I find everywhere in human enamel and the enlargement is due to the action of the acid which is formed beneath the bacterial plaque.

statement as this one sees that, for the moment at least, its author was totally oblivious to or forgetful of the multitudinous facts all about him concerning the action of bacteria and lichens on all forms of stone, wood and even metal. But in his conclusion Professor Pickerill really admits all that is necessary to a rational understanding of the whole subject. He says; "Those circumstances which favor

the formation of bacterial plaques upon the teeth also favor the lodgment of carbohydrates." Exactly so, as I have already pointed out, it is *that* concatenation of circumstances that produces the condition most favorable to decay. The mat-like mass of the plaque

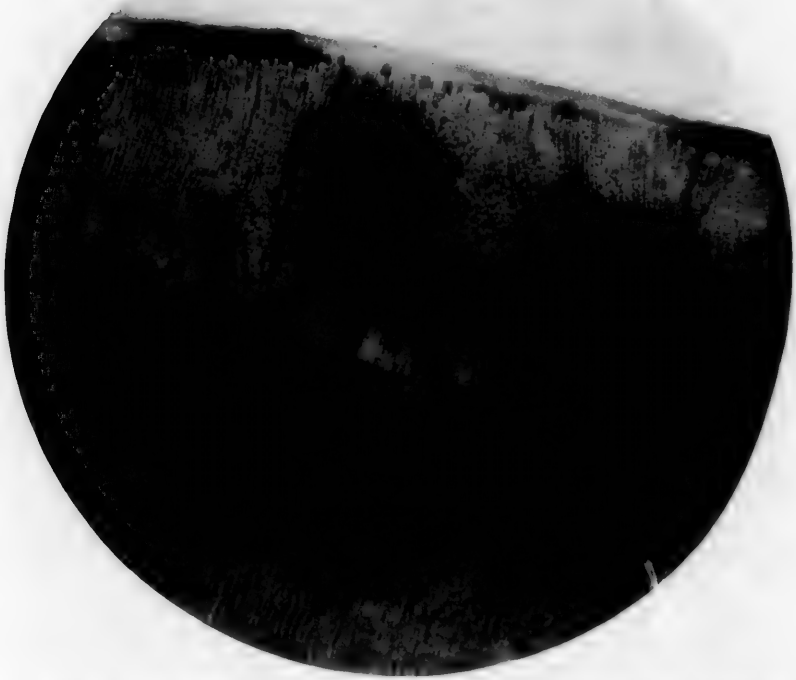


FIG. 56. From section shown in figure 52. The nitrate of silver stain reveals the action of the acid of decay throughout the entire thickness of the enamel.

sucks up and holds the carbohydrate material just where the acid-forming bacteria can act upon it and deliver the dissolving substance on the surface of the enamel. We see the evidence of this action when we come to examine sections of decaying enamel under the microscope.

I have tried a great many methods of producing dissolution of enamel by the action of acids, in the absence of the bacterial plaque, and in no instance have I succeeded in producing anything resembling the appearance of decaying enamel beneath the plaque. It may be that forms of decay are taking place in the absence of the bacterial plaque, but I greatly doubt this because I have never failed to find it when decay was occurring. The very great difficulty in preserving the plaque in preparing sections for the microscope is, I believe, the main reason for scepticism regarding its presence and action.[4]

In Dr. Howe's most recent paper, as published in the January issue of the *Journal of the National Dental Association* for this year, I note that he concludes that microörganisms are not the causative agents of decay. I understand that this opinion is entirely founded on his failure to produce decay in the teeth of animals by feeding them microorganisms isolated from caries in human teeth. This failure is not in the least surprising but I do not think it is any warrant for his conclusions. In fact, I should expect that a human being, immune to dental caries, might take quantities of acid-forming bacteria into his mouth for an extended period of time without any ill effects. As I have already remarked, it is highly probable that such bacteria may always be found in the mouths of immune persons. And in animals, especially the rodents, we are a long remove from the conditions which constitute immunity or susceptibility in a human being. Let us not confuse similarities with identities. The fact that profound disturbances of metabolism and even complete dissolution of the hard tissues of the teeth of guinea pigs may be produced without any aid from acid-forming bacteria, if such is really the case, does not warrant the conclusion that dental caries in human beings may not be produced by microörganisms. There is almost overwhelming evidence that caries in human teeth is so produced.

Nature often has many ways of producing conditions which, to the unaided eye, are similar in appearance but profoundly different when seen with the eye of the microscope. We shall find that this is the case when we compare the degenerate teeth of Dr. Howe's guinea pigs with carious human enamel. Let this not be interpreted as any

[4] I have reason to doubt if there is a single good specimen showing the bacterial plaque *in situ* in any dental college in the United States.

FIG. 57. The enlarged canals in decaying enamel are here very finely shown stained with nitrate of silver. Magnified about 1,500 diameters. I am uncertain whether the small black spots everywhere seen in this photograph are bacteria. That subject is being investigated.

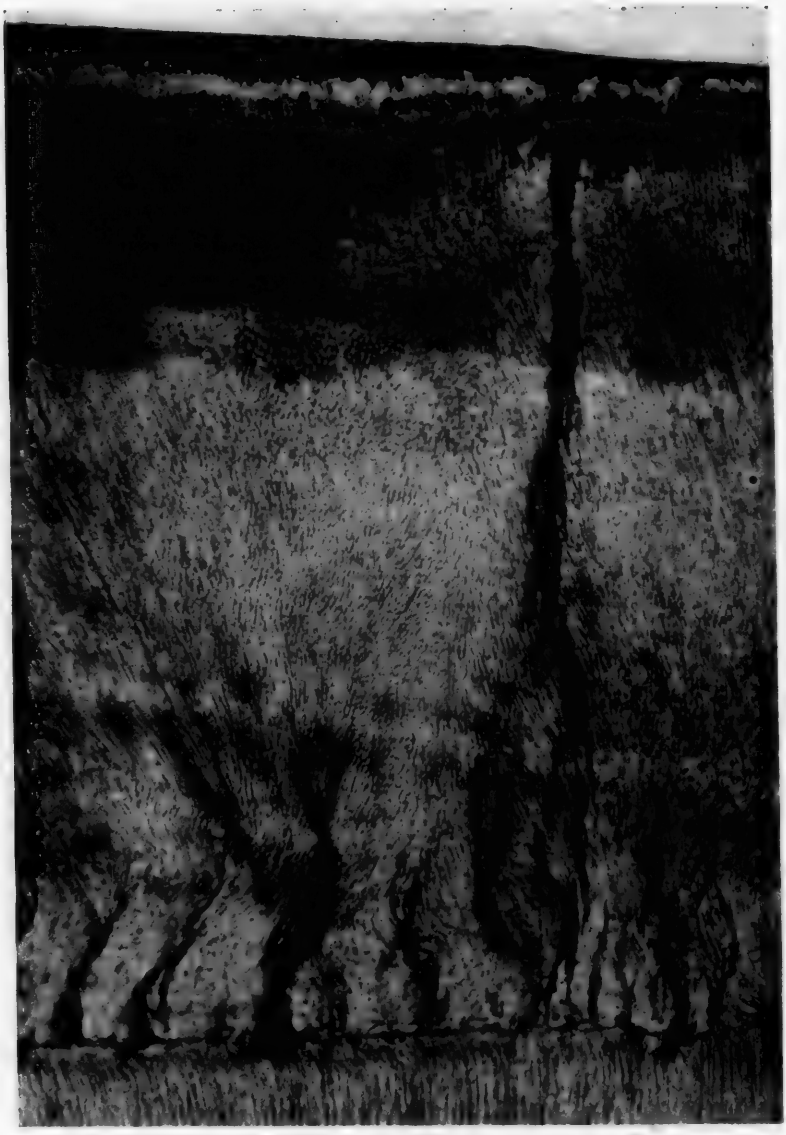


FIG. 58. Section from human tooth showing early stage of caries of enamel. The section is cut somewhat obliquely to the course of the enamel rods and stained with nitrate of silver solution. The black spots in the upper portion of the section show the manner in which the acids of caries penetrate the enamel beneath the bacterial plaque. This section also shows the spiral arrangement of the enamel rods and that a stain, entering the enamel from the dentine, always penetrates the center of the spiral first and then spreads out following the course of the rods.

disparagement of Dr. Howe's work. I will repeat here what I have more than once said to him, that I consider the sort of work which he is engaged in as of greater importance for the welfare of humanity than anything else that is being done in the field of dental

FIG. 59. In this specimen the acid of decay has penetrated the entire thickness of the enamel before there is any breaking down of the surface into a cavity. The action of the penetrating acid has produced a profound reaction in the dentine.

research. I am merely speaking a word of caution as to hasty conclusions before the facts of the case are all in.

I herewith present evidences of the dissolving action on the enamel of substances beneath the bacterial plaque. I have never seen such appearances in dissolving enamel when the plaque was absent. Until

some one brings forward proof that such appearances can be produced when the plaque is not present I must hold to the opinion that it is an essential feature in caries of enamel.

Pickerill says that bacterial plaques are found on the teeth of animals but he does not state whether wild or domestic animals were the subjects. I have never found any trace of a bacterial plaque on the teeth of any wild animal, but I should expect that microörganisms might be found attached to the teeth of animals whose normal powers of resistance had been impaired from any cause. Moreover, as he himself says, the presence of a plaque does not necessarily mean decay. (Study *figs. 52* to *61*, carefully.)

GENERAL CONSIDERATIONS

I have rapidly and briefly passed in review three pathological conditions of enamel, one showing the effects of vitamin-free food, one the result of inherited syphilis and the other dissolution of enamel beneath the bacterial plaque. Are these conditions in any way related? Is there any condition of any part of the organism common to all of these pathological changes?

I have pointed out the striking similarity of appearances in syphilitic enamel and that produced by excluding vitamins from food. Now there is a considerable and a steadily increasing body of evidence that certain disturbances of what is called the endocrine balance will produce very decided effects in calcium metabolism. Is there, then, an intimate relation between the presence of vitamines and the hormone products of the ductless glands?[5]

Professor Cobb, author of "The organs of internal secretion," has reached the conclusion that dental caries is one result of thyroid and parathyroid deficiency. It is also the opinion of MacCallam that calcium metabolism is controlled by these glands. Dr. Louis Berman

[5] Since these lines were written, in fact only the day before yesterday, the last issue of the *London Lancet* (January 7, 1922) reached me. This number contains a notable article, by a French physician, Dr. Pierre Vallery-Radot, on "The rôle of heredity in thyroid pathology." Keeping in mind what I have just pointed out concerning the similarity of syphilitic enamel to that caused by excluding vitamin C from food, Dr. Vallery-Radot's remark that "syphilis is at present one of the most evident causes of thyroid affections" is especially significant in this connection.

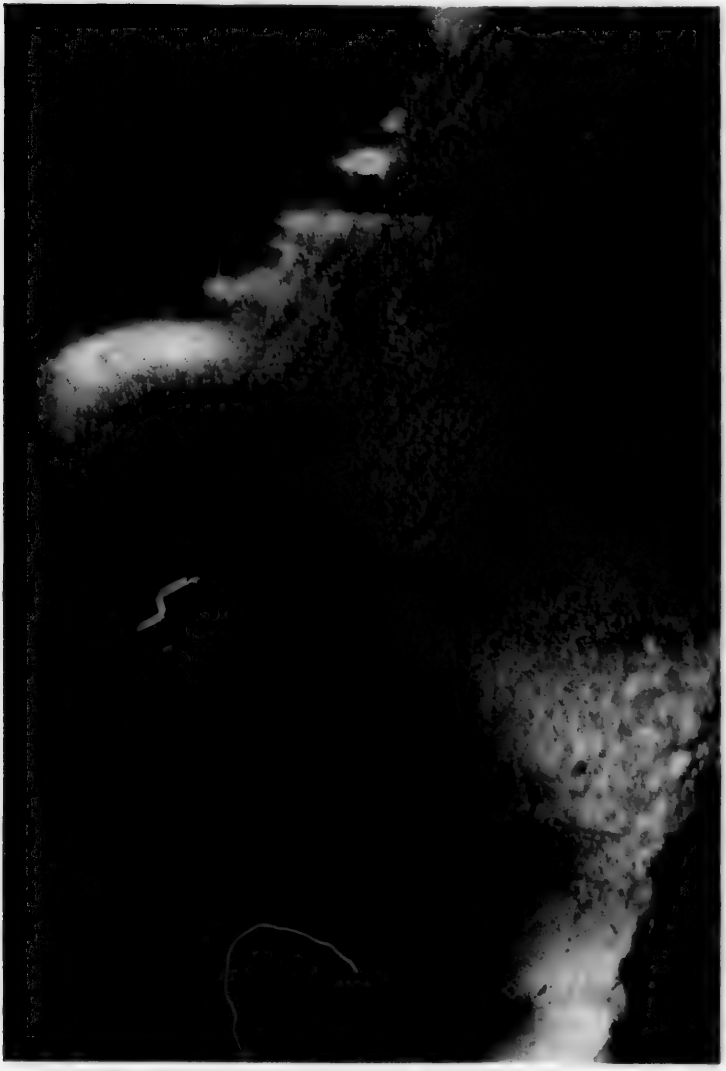


FIG. 60. The whole process of the early stages of decay of enamel is very finely shown in this specimen. The nitrate of silver stain has not only penetrated the enlarged canals but is diffused throughout the substance of the decaying enamel.

says that a conspicuous consequence of the removal of the parathyroids is failure of tooth development, particularly the enamel. Mr. F. W. Broderick, in a very interesting and suggestive paper read before

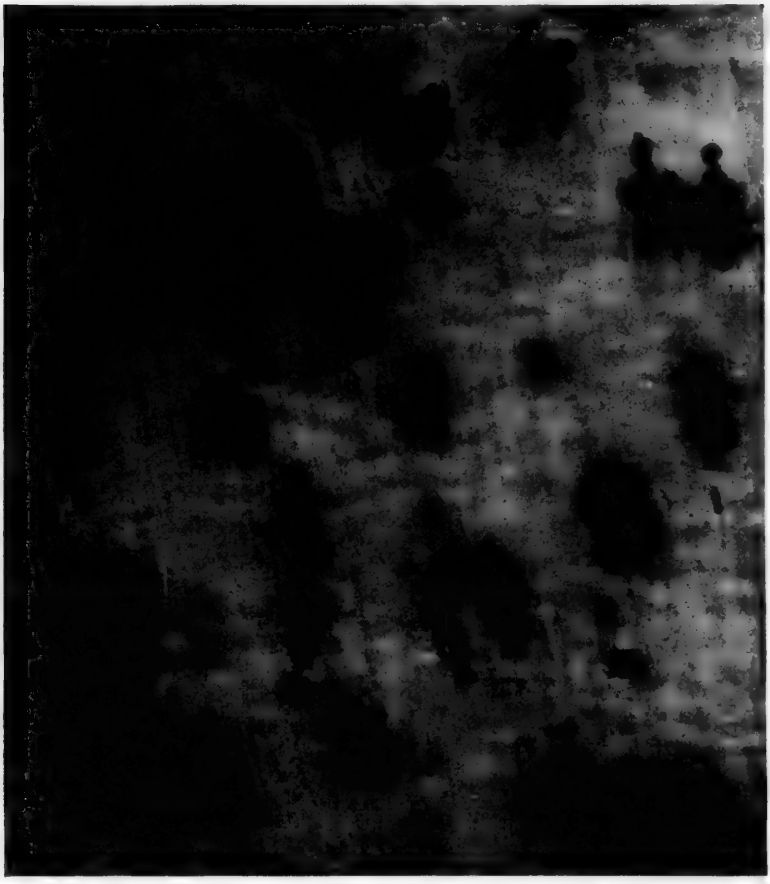


FIG. 61. Shows later stages in decay of enamel just previous to the commencement of the formation of a cavity.

the Royal Society of Medicine of London, on November 28, 1921, under the title, "The endocrine factor in the production of immunity and susceptibility of the teeth to caries," says that the body store of

calcium in health is preserved by the endocrine apparatus and that when the endocrine balance is upset one result is calcium starvation with diminished calcium index in the saliva and that this condition directly promotes caries. Dr. H. W. Vines in a recent communication puts forward the hypothesis that all acute and chronic toxic states may react harmfully upon the parathyroid glands causing a disturbance in calcium metabolism and a decrease in tissue resistance.

The work of McCollum, Hopkins, Funk, Mellanby, Howe and others, has demonstrated that the fat-soluble A vitamin is particularly concerned in the calcification processes of bone and teeth. Mrs. Mellanby, speaking of the results of her series of experiments on 300 puppies which extended over some six months, says:

> I am convinced that it is a dietetic factor, namely, vitamin X, which increases the amount of calcium retained in the body to build up bones and teeth, although of course this does not preclude its action being indirect and by way of the endocrine organs. Does this vitamin, then, have a specific action on the endocrine glands controlling calcium metabolism?

Here is a subject of the deepest interest to dentistry awaiting further investigation.

DISCUSSION

Arthur H. Merritt, D.D.S., in the chair. Introduction: At this first public meeting of the New York Section of the International Association for Dental Research, it is a great pleasure to welcome as our guests tonight so many of the members of the dental and medical professions who have journeyed long distances to meet with us, among them, Professor Arthur Hopewell-Smith, of the Dental School of the University of Pennsylvania; Professor George Alfred Bates, of Tufts College; Dr. Jacob Gutman, founder of the Brooklyn Diagnostic Institute; Dr. Herbert D. Pease, a representative of the profession of bacteriology; William K. Gregory, of the American Museum of Natural History and author of that masterly book on the Origin and Evolution of Human Dentition; Dr. Clarence J. Grieves, of Baltimore; Drs. Guy S. Millberry, Edwin H. Mauk, Robert E. Keys, and William H. Hanford, of San Francisco; Dr. Abram Hoffman, of Buffalo; Dr. William Rice, Dean of the Tufts College Dental School; Dr. Alfred P. Rogers, also of Boston; Dr. Bain, a representative of the Faculty of the Dental School of Georgetown University; and Dr. A. J. Brown, Dean

of the Dental School of Howard University; to these, and all others who are here tonight, we extend greetings and a most cordial welcome. It is an inspiration to have so many distinguished members of the profession with us. It speaks well for scientific dentistry that so many earnest men prove, by their presence, their interest in dental research.

It is also a very great pleasure to welcome, as our essayist tonight, one of the most distinguished investigators in dentistry of this generation, a man who has placed dentistry under lasting obligation to him for what he has already accomplished as a research worker. It is altogether fitting that our first public meeting should be addressed by one so well qualified to speak to us; Dr. J. Leon Williams, of London and New York.

Arthur Hopewell-Smith, Sc.D., L.R.C.P., M.R.C.S., L.D.S. (Philadelphia): Congratulations must first be extended to the officers of this Section of the new Association in the election, as its first Chairman, of Leon Williams, whose name and fame it is unnecessary to extol for his contributions to dental science in general and to the study of enamel in normal and abnormal conditions in particular. He is the greatest living exponent of the histology of this tissue. The dental profession must also be congratulated, in that, after a silence of fifteen to twenty years, he has again gone back to his *premier amour*, and given us, as he puts it, a "preliminary report" of his recent investigations. I am voicing a common sentiment when I say that the profession wishes him many years of fruitful labor in the elucidation of problems of anatomy, physiology, histology and pathology. I have to thank him personally for transmitting to me a copy of the address which he has just delivered, and I feel happy at being present on this occasion.

The solution of problems of anatomy are of paramount importance, for on an accurate knowledge of the histology of a tissue depends the precise conception of its functions and the abnormal conditions which it may undergo. In the dental tissues the difficulties of preparation and of staining are great, but greater are the problems of interpretation. The tissues vary so considerably, the reagents used in bringing out their minute structure frequently are so very elusive and uncertain in action, that it is easy to make erroneous deductions and thus spread "false doctrines, heresies and schisms." One need only give two illustrations. The results arrived at in using adjective stains for the nervous system, such as the methods of Golgi, Ramon y Cajal, Beilshowsky and others cannot always be relied on. Sometimes impregnation methods *do* answer successfully their purpose; at other times they fail completely, or act in various ways,

and so end disastrously for the achievement of the object for which they are primarily intended.

A word of warning must be uttered here in connection with this question of staining the dental tissues. The results from using the injection methods of von Beust for instance—where coloring material is injected under high pressure into the dentine and enamel are, and must be, from the very nature of the method, unreliable. The rules of microscopy must not be violated; nor may the science of optics be played with, in the examination of enamel by ultra-violet rays or polarized light, and ponderous conclusions based thereon.

Now the chief object of this investigation, I take it, has been to determine whether enamel contains organic material or not. But as Leon Williams says, "much more work remains to be done before a definite conclusion can be reached." The presentation of this question is therefore not yet complete. And it is clear that he is at present content to deal with collateral subjects having a more or less direct bearing on the main issue.

Enamel is said by some histologists to contain organic material from the way it behaves under the influence of stains. Because gold chloride, to take one example, sometimes stains the rods, therefore the rods must contain stainable material—protoplasm—is the oft-repeated argument. It does not necessarily follow. For it is perfectly easy to stain substances which admittedly are entirely inorganic, like marble or stone, by surface affinity, allowing the coloring fluid to remain for a varying period of time on the surface. But no one declares that marble contains protoplasm or fat or cartilage, as has been claimed for human enamel.

In the London address to which allusion has been made, I stated that human enamel is a dead substance. I adduced biological evidence to prove the point, and as one of those who heard the paper and subsequently discussed it, remarked, the teaching would appear to be a sound one. I shall not further refer to it here, but confine my remarks to those points in the present paper which seem to require them.

The structure of tubular enamel has been largely discussed, and again brought before us tonight. In some animals may it not be a modified dentine, and not enamel at all? I am not concerned in dealing with the histology of this. But I am concerned with the employment of so primitive a tissue, as a means of ascertaining the real nature of enamel as it occurs in man. Comparisons are notoriously odious, and too much stress must not be laid on the minute dental anatomy of those orders of animals such as the rodents and insectivores and particularly the marsupials, the

lowest and most aberrant of the *mammalia*, when dealing with problems of structure of the higher orders of the vertebrates. This objection does not hold in connection with a study of the teeth of the *primates* or even *carnivora*. Further, the structural features of the dental organs of the sharks and rays and their sub-orders, with their apparently tubular enamels, still more remote in their evolution than other ancient forms as exhibited in the marsupials, must be regarded only in the light of accessory knowledge, and of little intrinsic value where human enamel is concerned.

It seems to me that the question of the structure of human teeth should be devoted more to the histogenetic, physiological, physical, and clinical sides, and less to the phylogenetic side. The chemical aspect must not be ignored, though I must admit I would sooner rely, for progress of knowledge, on the deductions derived from a study of the former than of the latter. In addition, it must be remembered that most of the investigations of dental histologists have been made, as far as human tissue is concerned, on teeth which have been removed from the mouth, for some reason or other—the results of "caries," diseases of the alveolo-dental periosteum, or individual malposition. In each of these instances, material believed to be "sound" is most probably abnormal—even the fact of malposition in the dental arches postulates atypical variations of form or structure.

If the multitudinous variations to which teeth are peculiarly subject could be eliminated, more clarity would follow. Teeth are unique in this respect. Speaking from the larger standpoint, the histology of the tissues and organs of the body, *e.g.*, of the cardiac muscle fibres, of the splenic cells, or renal structure, is universally constant. It admits of but few variations. But not so the teeth. The behavior of different sections of the same piece of tissue, similarly treated and stained in an identical manner, presents numerous deviations from what one may regard as a type. Hence it follows that, however much I may regard the investigation and examination of tubular enamels as important in shedding light on the evolution of dentines and enamels, contributing to our knowledge of the evolution and biological problems associated particularly with those animals which possess them, to my mind this importance becomes almost and perhaps entirely nugatory when applied with equal force to human enamel, and dentine and cementum.

The whole question of the constitution of human enamel must be considered from the point of view of the study of normal material, its cellular origin, its method of formation, and the larger principles which underlie the science of function. As human enamel is of ectodermic origin; as it

is nothing more nor less than a beautiful calcified secretion; as it is developed in a centrifugal direction; as it fulfills a rôle similar to that of the stratified squamous epithelium of the body generally, *viz.*, that of protection; as it cannot respond, after completion, to the effects of disease in the form of inflammation, or of new growth—in a word, as it reacts to none of the fundamental principles of what we call "life," it cannot be considered living material, and as such cannot normally contain any organic constituents. Frequently defective in its make up, it may present to the chemist or biochemist some traces of organic substance: but these are, surely, subject to enormously great variations, and really only of academic and not of biological or histological interest. They do not confirm a statement or opinion of the histologist, but serve rather to embarrass him in his search for facts as revealed by the microscope, and perhaps even to produce stubborn mental astigmatism.

Of this I am convinced: enamel is much simpler in its histological constitution than some writers, with their "wings" to the enamel rods, "dividings and branchings," etc., would have us believe. For the manner of formation of these has not been explained, and to my mind admit of only one explanation, *viz.*, faulty technique. The belief that normal enamel passes through a stage of tubularity and presumably contains organic material is certainly entirely conjectural.

Dr. Leon Williams' suggestion that the inner ameloblastic membrane forms the inner layer of Nasmyth's membrane is new and interesting. When he speaks of "an outer layer of calcified or cornified cells," however, he is not describing this external layer as found in man. For here the cells are of the simple squamous epithelial type, in no sense calcified, partly calcified, or cornified. They are easily stained, very easily detached, and measure 12 by 25 microns. My observations in this matter corroborate those of Professor Paul, and tend to show that it is the partial calcification of the ameloblasts in man, which produce the inner translucent layer of the membrane, for the hexagonal or pentagonal markings seen through its surface correspond in size with those of the enamel rods. The epithelial cells of the outer layer have much greater dimensions, and are obviously not amelogenetic.

Regarding the enamel organ and its vascular or non-vascular supply, I may state that in addition to the evidences we have heard and read, I yesterday examined some sections of enamel organ of the white rat which had been prepared by Drs. Addison and Appleton of the University of Pennsylvania, and there was the clearest possible indication of the passage of blood-vessels into the stellate reticulum of the enamel organ of the

molars. Also this penetration was noticed and described by Bolk a few years ago. I have repeatedly observed it in the pig and kangaroo.

In conclusion, reverting to the question of the presence of organic material in human enamel, since seeing the wonderful photomicrographs, the thought has occurred to me that the "tubes" of enamel may be tubular spaces, or canals, or holes, different apparently from the dentinal tubes which have definite walls—the sheaths of Neumann. I presume that the terms used by Leon Williams, "fibres" and "tubes" and "canals," are synonymous. I have no difficulty, if we accept this view of these developmental defects—if such they are—in understanding the reasons why some observers have stated that organic material is present in enamel, and that gold chloride and other stains can be easily precipitated in these canals.

Jacob Gutman, M.D., Phar.D., F.A.C.P. (*New York*): I am greatly indebted to the President of the Association, Dr. J. Leon Williams, for the cordial invitation to attend this meeting, and the privilege of listening to and discussing his most scholarly address. As a medical man, I am not in a position to comprehend fully all that was presented this evening, but could not fail to be impressed by the immensity of the subject, the enormous efforts and energy expended by the author in its preparation, and the invaluable data offered this evening, which is bound to be of the utmost benefit to both professions—medical and dental.

The first three features mentioned in the address—first, the effect of vitamine-free food upon the teeth and their enamel; second, the observations on inherited syphilis, and the histo-pathology of teeth; and third, the bacterial dissolution of enamel—are of great interest to me and impressed me vividly, but I shall not endeavor to discuss these features, as there are present, undoubtedly, others more competent to do so. Yet I cannot pass without remarking that the extraordinary similarity of histopathogenetic processes observed by the reader of the paper in the enamel of animals fed with vitamin-free food and individuals with inherited syphilis, as shown here tonight, is a remarkable discovery deserving further study and investigation. I shall confine myself to a few remarks upon the fourth feature, the endocrine, mentioned in the paper—the association of endocrine disturbances with the state of the teeth, a subject which has interested me greatly during the past few years.

The time has now come when even the medical man is beginning to recognize the tooth as an organ, and to realize its important functions and far-reaching effects upon the organism. If the tooth is an organ, then

it must necessarily conform to the same laws governing all other organs and be subject to the same influences of metabolism as exercised by the endocrine glands upon the other organs.

That the endocrine glands are the chief controlling forces of metabolism cannot be further denied. There is no evidence that any other organ in the animal economy exerts such enormous influence over the metabolic changes as do the endocrines. There are no instances in the pathology of other organs comparable to the changes induced by pathological affections of the endocrine glands, as exhibited in acromegaly, myxedema, eunuchoidism, cretinism, etc. Another thoroughly established fact is that the endocrines control the metabolism of every important body constituent —the proteins, fats, carbohydrates and salts, among which calcium, which is of particular interest to us tonight, is one. Faulty calcium metabolism must have its effect upon teeth, whose welfare is so much dependent upon this substance, constituting as it does a very appreciable percentage of its contents. Calcium is not only required by the teeth but it is an absolute and indispensable constituent of every cell. Without calcium no cell can live, as was demonstrated long ago by Jacques Loeb in his experiments with unicellular organisms. Hence its introduction into the body, its assimilation and absorption, are of enormous importance to the welfare and maintenance of the whole organism. Whenever interference occurs with any of the phases in its assimilation, from whatever cause, the organism is bound to suffer—the teeth and bones, which require the greatest amount of calcium for their maintenance, the most. Furthermore, when calcium deficiency occurs during the developmental stage, as during fetal life, in infancy or early youth, the effects of such calcium impoverishment are even more marked in the teeth and bones, examples of which are met with daily in rickets and similar constitutional disturbances.

The calcium is imbibed in food, is then taken up in an absorbable form by the great absorptive area of the gastro-intestinal tract, and transported through the blood where it is retained at a constant percentage, varying but little under all circumstances, to every cell in the body. The excess, if any, is stored in the bone, its storage warehouse, or eliminated chiefly in the faeces and less in the urine. It is an established fact that the parathyroid is the primary influential factor in calcium metabolism. It has been conclusively proven that in all conditions accompanied by anatomic or pathologic changes in the parathyroid, there occurs a change in the calcium content of the blood. Thus, in rickets, tetany, adynemia, convulsive seizures, laryngismus stridulus, spasmophilia, and similar

conditions characterized by an increased muscular tonus, now deemed to be of parathyroid origin, there is always a disturbance in the calcium blood balance. Whether other endocrine glands, such as the thyroid or pituitary, for which some investigators claim such function, are concerned also with calcium metabolism, is a debatable question; but that the parathyroid is to be credited with marked influence on calcium metabolism is now well established, as numerous experiments on animals and clinical observations on man have fully demonstrated. It may thus be safely assumed that the calcium content of a tooth depends greatly upon the calcium metabolism of the individual, which in its turn is regulated and dependent upon the state of his parathyroids.

As to the many other features of the teeth, such as their shape, their color, position, durability, period of eruption, tendency to decay, etc.: these may beyond any doubt be ascribed to the domination of the endocrine glands. I need not, at this time, go into detail, as your eminent colleague, Dr. Chayes and my friend, Dr. Kaplan, have already, in their communications, very ably brought such association to your attention. Nor does the time permit me to elaborate upon several other features which Dr. Williams has presented in his paper on matters pertaining to calcium metabolism, the teeth, and constitution. I wish, however, before closing, to recall to your attention the fact that it is of the utmost importance, if it is desired to understand the whys and wherefores of the condition of the individual's teeth, to study him from the endocrine standpoint. The state of the teeth is so closely associated with the general condition of the individual, which again is so much dependent upon his endocrine balance, that neither condition can be studied intelligently without consideration of the other. Pathology closely follows abnormal function. Pathological physiology is the result of disturbed endocrines. The knowledge of these illumines nearly all questions arising in the analysis of a case.

I trust the time may not be far off when the numerous problems propounded by Dr. Williams in the course of his address this evening will be elucidated, but this we may only hope to attain through closer coöperation between the dental and medical professions, which is stimulated greatly by such meetings as this.

George A. Bates, M.S., D.M.D. (Boston): Assuming that the structure that Dr. Williams finds in marsupial enamel is organic, the question of sustenance arises at once. The fact that all sustenance in the body must proceed from elements contributed by the blood gives rise to a second

question: How does the blood reach that organic matter? Two sources only can be considered, since these are the only ones that in any way ever come into sufficiently intimate relation with the enamel. One is the enamel organ; the other, the dentine. The first must be ruled out since it ceases to exist as soon as the enamel is fully formed. The only remaining source is the dentine. Under these conditions how can the hypothetical organic matter come into contact with the blood, since no blood exists in the dentine? The nearest approach to a blood supply in the dentine is its contact with the pulp. The pulp not only contains the blood vessels but also the connective tissue necessary to lymphatic circulation, upon which the process of metabolism depends. This aspect of the problem narrows the issue to metabolism and justifies the further question: what is metabolism?

The problem of metabolism is a complex one. From a superficial point of view it is simply the process of carrying nutriment and oxygen to the cell, where they are utilized in liberating energy and supplying material for the building of new protoplasm, and the elimination of waste. Thus stated it would seem to be a very simple process, but when all the known facts are considered it assumes a much more complicated character. Not only must we consider the process by which the blood delivers its load of food products and oxygen to the cell, but also the transformation of non-living into living matter and the subsequent disposition of waste. This phase of the problem must involve three constituents: a cell, blood, and connective tissue; the last to act as a vehicle to convey the blood to the cell which receives it and carries on the subsequent operation. The only element in the dentine bearing the slightest resemblance to or having any association with a cell is the dentinal fibril, which is a process proceeding from the odontoblast situated in the pulp. The only way this fibril can possibly reach the enamel is through the dentinal tubule.

Does the dentinal tubule penetrate the enamel? If so, how can it carry on the process of lymphatic circulation in the absence of cells in the enamel—and this is the only possible way for metabolism to occur. It has been claimed that there is a circulation in the dentinal tubule. If this is true, whence comes the circulating medium and where does it go? If it emanates from the pulp and passes through the tubule, returning again to the pulp, what purpose does it serve? It must be lymph, if it serves any good purpose in the interest of metabolism; and it is difficult to determine where the metabolism occurs since, with the exception of the odontoblast and its process (fibril), there are no cell elements in the dentine or enamel.

All the metabolism must occur in the odontoblast, since this is the

only cell involved. It is difficult to attribute metabolism in the enamel to this source unless the fibrils penetrate and everywhere permeate that structure; and, so far as I understand, all the evidence thus far advanced to demonstrate this fact is very inadequate and unconvincing.

Of what avail is a circulating material, if it does not reach every part of the structure to be acted upon? In all the sections that I have seen the so-called projections of dentinal tubules said to penetrate the enamel enter this substance only a very short distance and are so vague and indefinite that they may be interpreted as cracks in the enamel with the same propriety as the ultimate extremity of dentinal tubules. Under such circumstances it is difficult to determine how contact with the hypothetical circulatory medium in the dentinal tubule can be established for enamel.

There is, however, another phase to the problem. Dr. Williams is dealing with marsupial enamel which, as is well known, is very primitive. Only the monotremes are lower. Both of these groups are very low in the mammalian scale. It is true, as long ago shown by John Tomes, that among the lower vertebrates there exists tubular enamel in which more or less organic matter exists. Are we dealing here, in Dr. Williams' problem, with an expression of the phylogenetic process by which, in the upward progressive development in the vertebrate stem, the primitive conditions existing in the lower species have been projected into structures characteristic of the higher forms?

In the beginning of development, there is a quantity of organic matter in the mammalian enamel which, I believe, is subsequently completely calcified; and *remains of it, in calcified form, might be demonstrable in adult enamel.* Is this perhaps true of the lower vertebrate forms, but on a larger scale? It would be helpful and interesting to study these teeth during ontological development; also to follow such observations through a succession of forms in a phylogenetic series, if such a comparative inquiry were possible.

It is a question whether the enamel is a part of the tooth. It is developed from a different blastodermic layer than that of the dentine and cementum, and is plastered onto the already-formed dentine—and only for purposes of protection. Its origin is distinctly involved in the problem of the enamel organ, which Dr. Williams, the most competent observer in the world, just touched upon in his paper. When more is known on this complex problem, the solution of the present quest will be nearer completion.

(Dr. Williams's response to the foregoing discussion is covered by the succeeding paper.—*Ed.*)

MOTTLED ENAMEL, AND OTHER STUDIES OF NORMAL AND PATHOLOGICAL CONDITIONS OF THIS TISSUE[1]

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New York City

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I. INTRODUCTION

About three years ago I began a reinvestigation of the field of enamel formation and structure which I covered some twenty-eight years ago. I was led to do this because of the statements of Pickerill and others as to the permeability of enamel, and differing degrees of consolidation and hardness of this tissue as shown in different teeth. This new investigation has resulted in two things: a confirmation of most of the views which I had published on the formation of enamel and a considerable modification of my former opinions as to the structure and nature of fully formed enamel. I had formerly agreed with Black, Hopewell-Smith, and others, that normal human enamel was impermeable to stains. The evidence now available makes such a position entirely untenable. As histological evidence of the permeability of enamel must depend almost entirely on the results of our efforts to stain it, a few general remarks on this subject, as a preliminary statement, will be in order.

In what was, I believe, the last paper written by Dr. G. V. Black, in collaboration with Dr. Frederick S. McKay, on *mottled enamel*, he said: We cannot stain the body of normal enamel with any stain-

[1] This paper was read before the Ohio State Dental Society, at Cincinnati, on December 5, 1922, on the occasion of the presentation to Dr. Williams of the first Callahan Gold Medal.—(*Ed.*)

ing agent we know." I had said the same thing many years before. And then, later, came statements from a number of competent observers, notably Mr. J. Howard Mummery of London, Dr. C. F. W. Bödecker of Berlin, Caush, Von Ebner, Fleischmann, Von Beust, and later Professor Russell W. Bunting and others. In deciding to reinvestigate the structure of enamel I was influenced also by the work of Professor Gies, who succeeded in passing aqueous fluids through the enamel by way of the pulp chamber and the dentine and also from the surface inward. This work, which has occupied a considerable part of my time during the past two years, not only has confirmed the findings of other investigators as to the permeability of enamel but has also demonstrated (what was not clearly known before) the nature of the channels through which the circulation or passage of fluids takes place.

As I look back now upon my belief that enamel was an unstainable tissue, I am astonished and somewhat puzzled to know how I missed making the experiments which would have demonstrated my mistake. But my wounded vanity is somewhat soothed by the knowledge that in holding this belief I had the support of so acute and able an observer as my old friend, Dr. G. V. Black.

Let me say, then, at the outset, that I have not found any human tooth which is not permeable to stain in some part of its enamel structure. My method has been to stain the tooth in bulk before sectioning. I would not regard the staining of sections of enamel as a satisfactory method, in itself, of demonstrating the real structure of this tissue, although important corroborative evidence might be obtained in this way. When a stain passes from the pulp chamber through the dentine and enters the enamel, or when it enters and penetrates the enamel from the unbroken surface of the tooth, there has been demonstrated at least the possibility that solutions which might form chemical combinations may pass into the tooth in a natural way during its life period. I say no more than that the *possibility* of such a phenomenon has been demonstrated; but that is a decided step in advance of what has heretofore been the view of the majority of our most eminent dental histologists.

As the photomicrographs which I am to show you present a number of aspects that are new, and as these new views are due entirely to

staining effects, I think it will be well to say a few words more on this subject of staining. Perhaps I can best bring before you the full significance of these views of stained enamel, as compared with unstained specimens, by asking how much we should know today of tissue and cell structure if staining methods had never been employed. What should we know of the wonderful processes of mitosis, of centrosomes, chromosomes, asters, spindles and polar bodies but for the staining agents which have been used in demonstrating these features of cell changes? So it is with enamel, although in a less degree. As we shall see, staining has thrown a flood of light on the structure and condition of forming enamel, completely formed normal enamel and pathological enamel.

While a number of stains with which I have experimented enter enamel freely, notably eosin, indigo eosin, chloride of gold and nitrate of silver, I have found that a solution of silver nitrate (2 per cent to 5 per cent) greatly excels all others in its selective quality. Eosin and also gold chloride have the character of a diffused stain. The substance of the enamel rods appears to be stained about the same as the inter-rod substance. Not so with silver nitrate. This very markedly first stains the periphery, or a portion of the periphery, of the enamel rod. I shall speak of the significance of this later. In many instances the stain, if the action is continued long enough, will penetrate every part of the enamel. The nitrate of silver stain presents three distinct appearances. Occasionally, although not often, the stain will be, in some areas, pale violet. In nearly all specimens some areas will be clear orange and others quite black. Nitrate of silver marks every slightest trace of imperfection in the structure of enamel whether congenital or acquired. This agent reveals the fact that, while there are great variations in the permeability of enamel, there are but very few teeth that are even approximately perfect in the structure of this tissue if by that word "perfect" we mean impermeability to stain.

The investigations which I am about to describe embrace three distinct series of studies: mottled enamel; the enamel of fully formed but unerupted teeth; and the enamel of mature teeth in normal and pathological conditions. The striking and illuminating thing about these different studies is seen in the light that one throws on the others.

II. MOTTLED ENAMEL

Let us begin with our study of mottled enamel. Dr. Frederick S. McKay kindly placed in my hands nearly two dozen specimens of teeth showing this peculiar dystrophy of enamel. Dr. Black's very exhaustive article in *Dental Cosmos*, of February, 1916, deals with the macroscopic appearance of mottled enamel and also with its microscopic appearance under low powers. I shall not traverse much of this ground as it is hardly possible to add anything of importance to his excellent description, as far as he went. In one or two instances I find myself obliged to disagree with his conclusions, but on the whole this piece of work, like everything he did, exhibited acute observation and accurate description.

It will be seen, then, that the results of my own observations under low powers of the microscope agree substantially with Dr. Black's report except in so far as staining has modified those views by revealing new facts. Mottled enamel is an endemic dystrophy of this tissue, which seems to be confined largely to certain areas in the Rocky Mountain country, notably in Colorado, although specimens are found in various parts of the world. The teeth are characterized by a dead white or milky white appearance which is varied by brown and yellowish brown spots and bands. The condition of the enamel which produces this appearance varies from about one-fourth to the entire thickness of the tissue. In the majority of the specimens which I have examined the imperfectly formed tissue has been from one-third to one-half of the entire thickness of the enamel.

I select for my first study an upper cuspid which seems to the unaided eye quite perfectly formed except that it is paper-white in appearance. Using a thin disk, I split this tooth longitudinally through the center of the crown. I polish the cut surface of one-half and photograph it by *reflected* light under a power of about 10 diameters. The appearance is as shown in *fig. 1*. A white band extends around the outer part of the cut surface, occupying one-half and in some places more than one-half of the thickness of the enamel. Inside this white band, extending to the dento-enamel border, there is normally formed tissue. After being photographed by reflected light, the specimen is placed in a 5 per cent solution of silver nitrate for a

FIG. 1 FIG. 2

FIG. 1. Section of a superior cuspid, before staining. A characteristic example of mottled enamel. The specimen was photographed by *reflected* light. By this method the outer, abnormal part, *A*, of the enamel shows light, while the inner, normal portion, *B*, is dark.

FIG. 2. The other half of the tooth shown in *fig. 1*, stained with a weak solution of nitrate of silver and photographed by *transmitted* light. The light and dark areas are the reverse of those in *fig. 1*. The stain enters the defective enamel rapidly; but, if not exposed to the stain too long, it goes no farther than the line which separates the abnormal from the normal tissue.

FIG. 3 FIG. 4

FIG. 3. Section of a lower second molar, before staining, treated the same as *fig. 1*. In this specimen of mottled enamel the full thickness of the tissue is defective.

FIG. 4. The reverse of *fig. 3*. This specimen was treated like *fig. 2*, but stained with eosin. The stain penetrated the full thickness of the enamel in 90 minutes.

few hours. The cut surface is ground away to remove any surface deposit and a section is made, mounted in balsam and photographed by *transmitted* light with the same lens that was used in the first specimen (*fig. 2*). Here we have all the conditions of light and shade

FIG. 5. Section of a superior bicuspid treated the same as *fig.* 2. This specimen demonstrates the very significant fact that in all cases of mottled enamel it is only the later deposit which is abnormal. Unless the full thickness of the enamel is defective, which is *rare*, the inner half or two-thirds, which is the first deposit over the tips of the dentine cusps, is normal.

reversed. The outer band, which was white by reflected light, is now, by transmitted light, quite black. The stain has rapidly penetrated the paper-white area of enamel but has stopped at the beginning of the normally formed tissue.

I now cut a lower molar transversely at about the middle of the

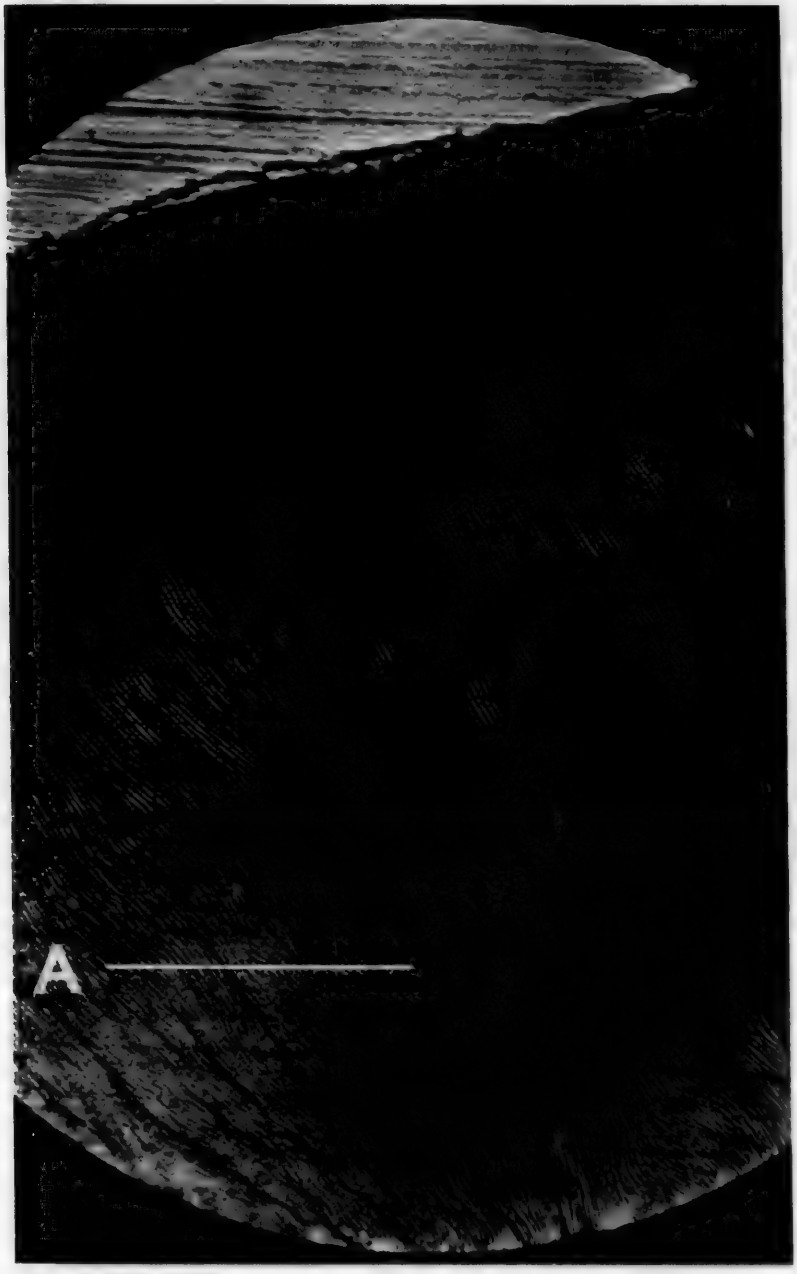

FIG. 6. Unstained specimen of mottled enamel. *A* represents the dividing line between the outer, abnormal tissue and the inner, normal part. Obj. 16, proj. oc. 6.

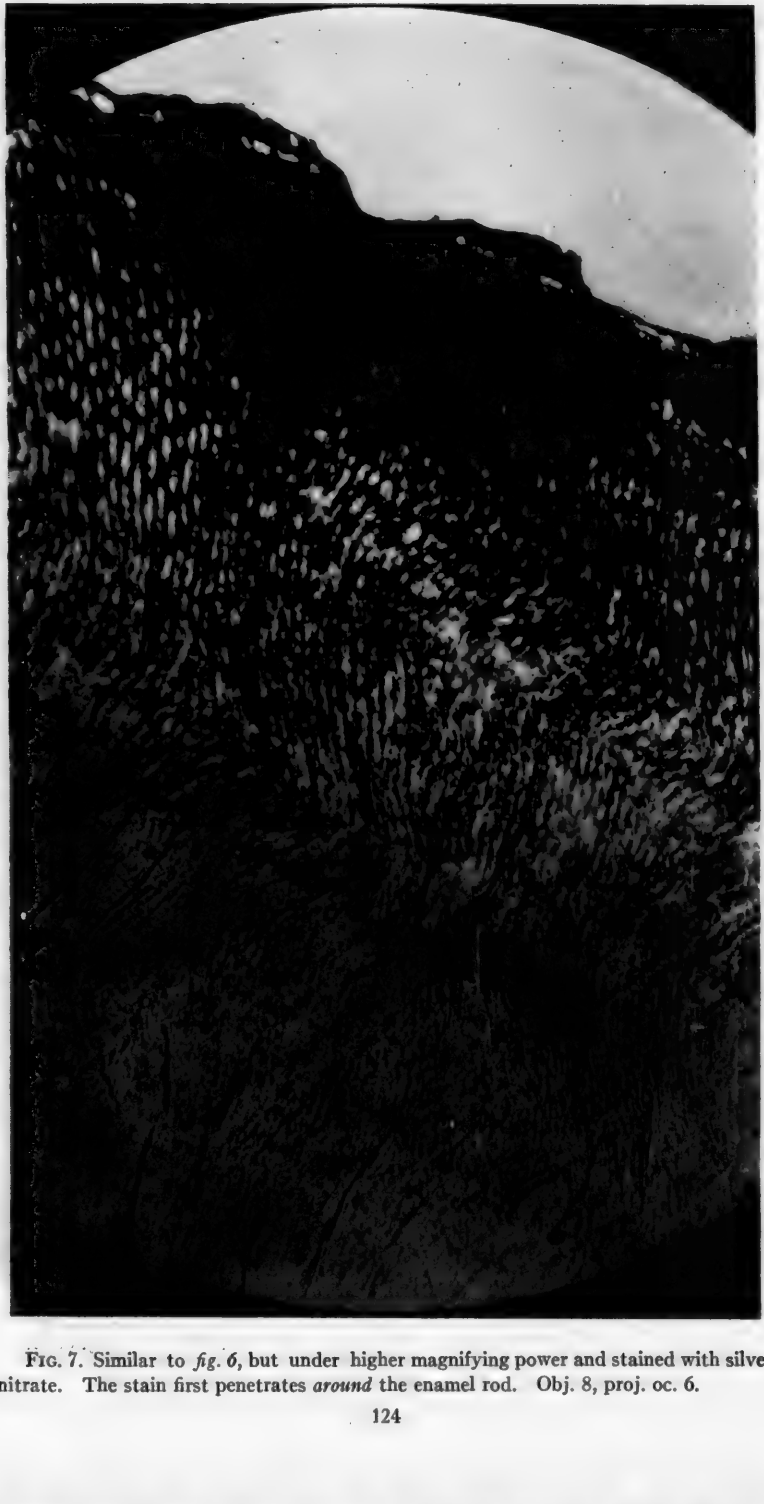

FIG. 7. Similar to *fig. 6*, but under higher magnifying power and stained with silver nitrate. The stain first penetrates *around* the enamel rod. Obj. 8, proj. oc. 6.

crown and photograph the cut surface as before. In this specimen it is seen that the entire thickness of the enamel is paper-white (*fig. 3*). After being photographed by reflected light, the specimen is placed in an alcoholic solution of eosin. The stain penetrated the entire thick-

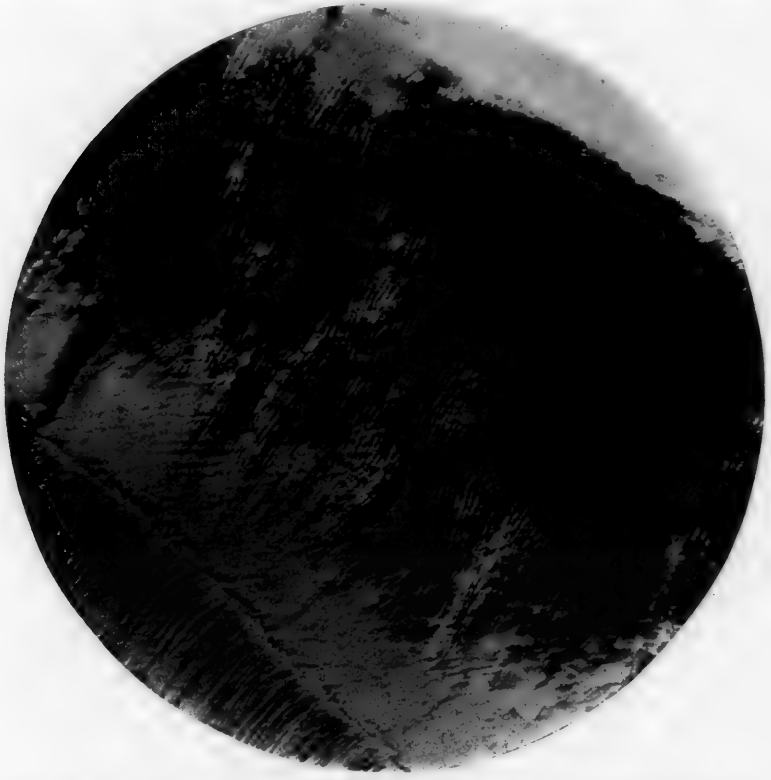

FIG. 8. A specimen of mottled enamel in which nearly the entire thickness of the enamel is defective. This specimen was stained with silver nitrate in bulk in 15 minutes. Obj. 16, proj. oc. 8.

ness of the enamel in ninety minutes (*fig. 4*). These specimens sufficiently familiarize us with the fact that stains will rapidly penetrate that defective condition of the tissue known as "mottled enamel."

Fig. 5 presents a section of a superior bicuspid treated the same as *fig. 2*. This specimen demonstrates the very significant fact that in

all cases of mottled enamel it is only the later deposit which is abnormal. Unless the full thickness of the enamel is defective, which is rare, the inner half or two-thirds, which is the first deposit over the tips of the dentine cusps, is normal. I have no suggestion to offer in

FIG. 9. A stained specimen in which the dividing line between the normal and abnormal tissue is very sharply differentiated. Obj. 8, proj. oc. 8.

explanation of this strange fact. Up to the present time all theories advanced, including the recent one put forward by Beust, completely fail to explain the fact to which I herewith call attention and which this specimen perfectly illustrates.

Let us now examine a few specimens under 16-mm. and 8-mm.

apochromatic objectives, which give medium magnifying powers. Our first is from an unstained specimen (*fig. 6*). As Dr. Black has very truly said, the untrained eye would hardly discover anything wrong in the appearance of the enamel here. But a similar tooth which has been exposed to nitrate of silver stain, and then sectioned,

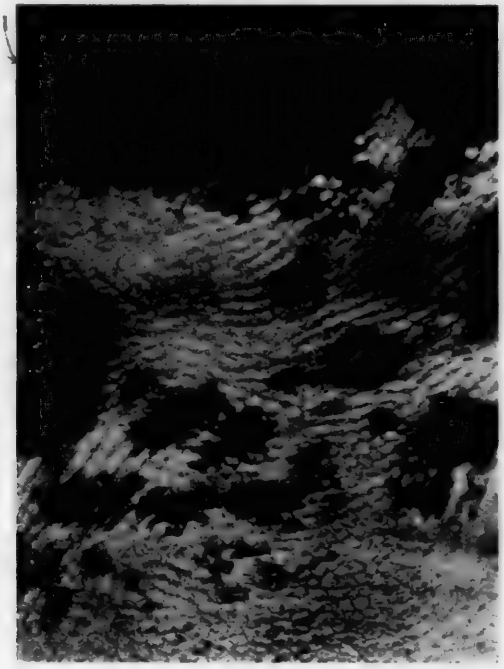

FIG. 10. Transverse stained section made near the border line between the normal and abnormal areas. It is in this location that we get our most instructive views of the exact nature of mottled enamel under high magnifying powers. (See *fig. 12.*)

shows the stain penetrating about one-half the thickness of the enamel (*fig. 7*). Usually the stain will penetrate the entire depth of the defective enamel in an hour or two, but some of the specimens which I am exhibiting were in the stain for only a few minutes. The object of these short exposures to a stain is to show the manner in which the stain penetrates the canals or open spaces in defective tissue. If the

specimen is left in the stain for any considerable length of time, it becomes so black that all detail is obliterated (*figs. 8* and *9*).

Exactly what is the character of this defective enamel which permits stains to enter so rapidly? To determine this we must examine oblique and transverse as well as longitudinal sections of the enamel rods under high-power objectives, which, with an ocular magnifying 10 diameters and various camera extensions, will give us magnifica-

FIG. 11. A very striking view of the sheaths of the enamel rods deeply stained with nitrate of silver. The surrounding normal tissue was so much overexposed that all detail is lost.

tions ranging from 600 to 3000 diameters. This reveals a whole new series of facts never before shown.

I have found my most instructive studies at the inner border of the stained areas, that is to say, just where the defective enamel merges into the sound enamel. *Fig. 10*, under a power of 500 diameters, shows these areas finely. The enamel rods are cut nearly transversely and we see at the first glance that the circumference of each enamel rod is the first part of the tissue to take the stain (*figs. 11* and *12*). *Figs. 13* and *14*, under a power of about 1000 diameters, give a very

striking picture of the condition of this defective enamel on the border line of the more perfectly calcified area. Please observe closely that

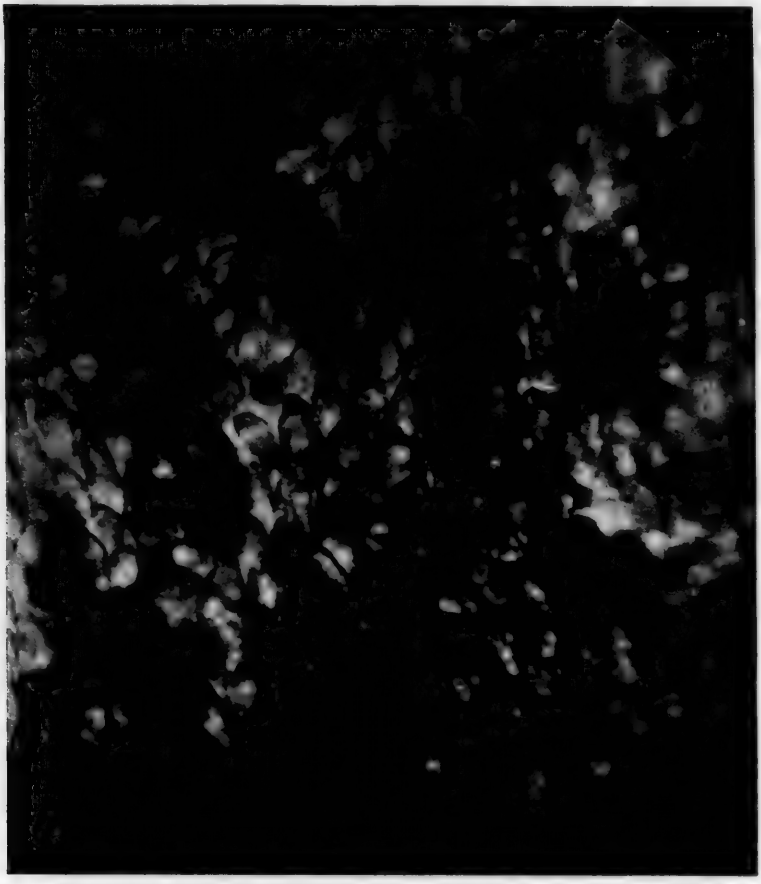

FIG. 12. Slightly oblique section of mottled enamel from the area shown in *fig. 10*. A sharply defined stained area is seen surrounding each enamel rod, and many canals along which the stain has penetrated. Apochr. obj. 3, proj. oc. 6.

not all of the substance between the enamel rods is stained. Each rod, as I have said, is very sharply outlined in black, as if a pen-line had been drawn around it. Now, the space between enamel rods

varies considerably in different specimens, but this sharply defined line is always the same in all specimens. As we shall see, it is quite distinct from the larger canals which we find, to a greater or less extent,

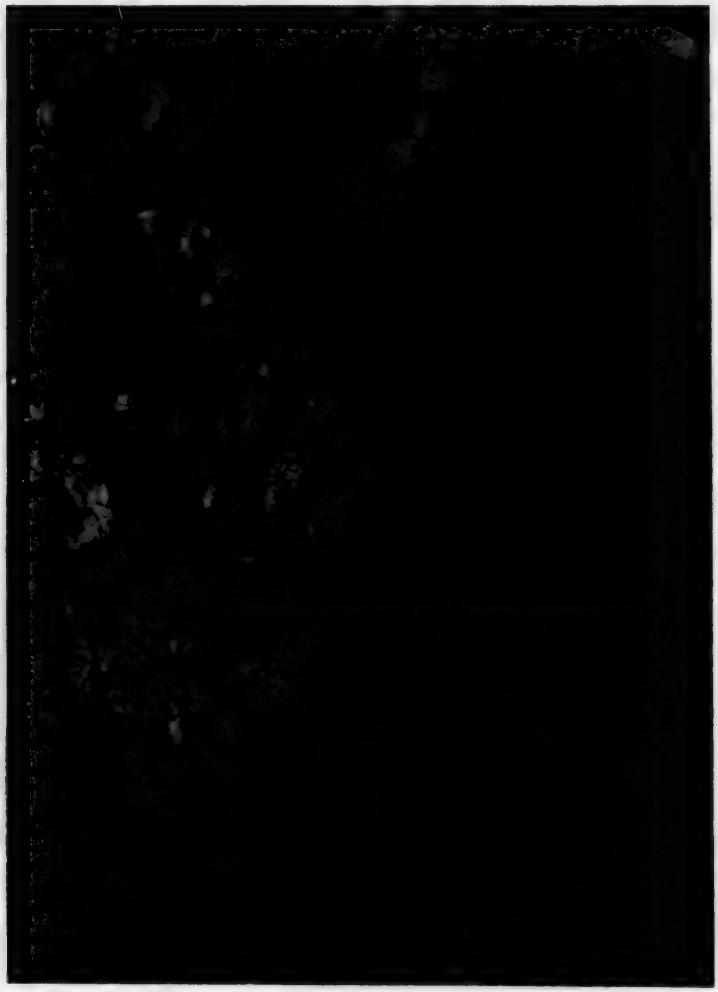

FIG. 13. Similar to *fig. 12*, but with stained defective area around certain enamel rods more sharply defined. This illustration alone completely upsets and refutes Beust's theory. Apochr. obj. 2, proj. oc. 6.

in all enamel. As we approach the more defective tissue, these sharply defined lines often become confluent with the larger canals. This condition is shown in *figs. 15* and *16* with all the distinctness of pen and

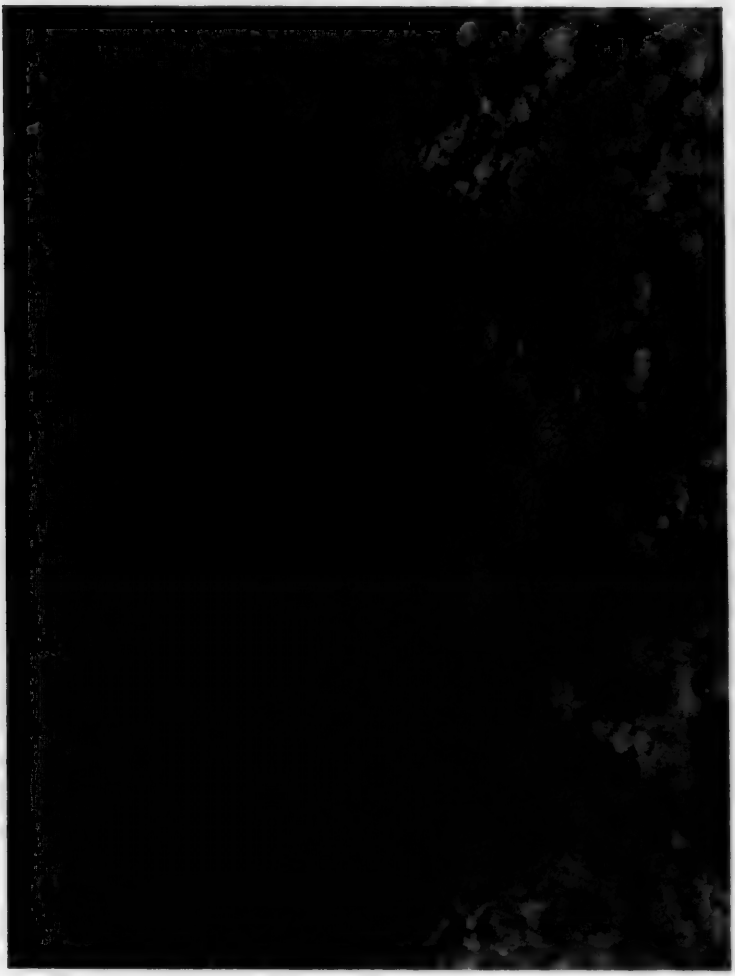

FIG. 14. From another specimen similar to *fig. 13*. The surface of the three foregoing specimens was slightly etched with a weak solution of lactic acid to bring out more clearly certain structural features of the enamel rods and their relations.

ink drawings. *Figs. 17* and *18* show mottled enamel, under very high power, near the border line of the imperfect tissue. The structure and

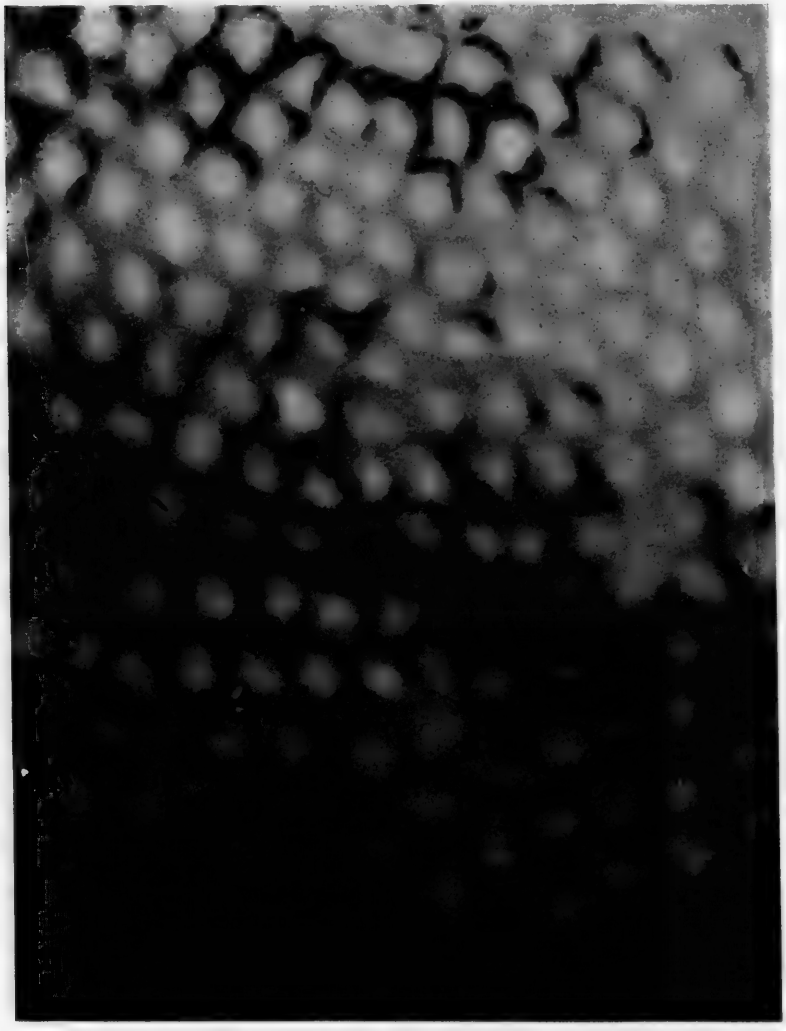

FIG. 15. This and the following (*fig. 16*) are photographs from highly polished specimens which were not etched with acid. The stain enters the sheath or space which surrounds each enamel rod and by way of larger canals.

relationships of the enamel rods are finely shown here, as well as the defective condition of the cement-substance. *Fig. 19* is from a specimen cut somewhat obliquely to the course of the enamel rods. The place where the rods are seen to change direction represents the border

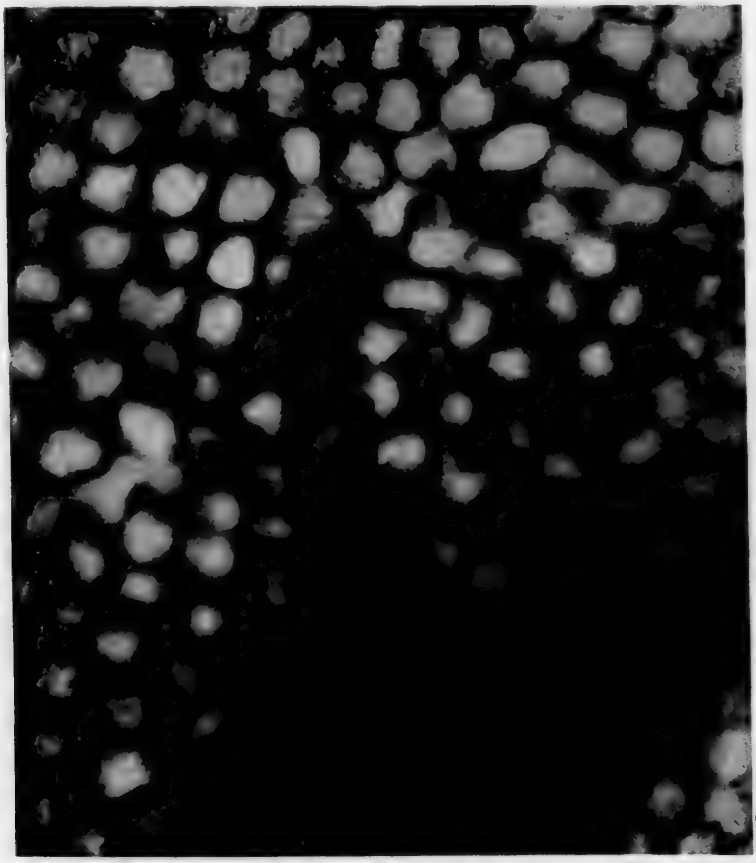

FIG. 16. In this specimen the differentiation between the normal, perfectly calcified tissue and the abnormal, imperfectly calcified tissue is very clearly shown. Just what the condition is of the stained area or sheath surrounding the enamel rods in the defective areas is not at present known. In dried specimens it seems to be largely a space containing nothing but air, but in the mouth it may be filled with a soft, gelatinous, imperfectly calcified matrix. (See *fig. 15.*)

line between the paper-white defective enamel and the more perfectly calcified tissue. There is a small area of sound enamel shown on the left of the photograph. Immediately to the right and above this we

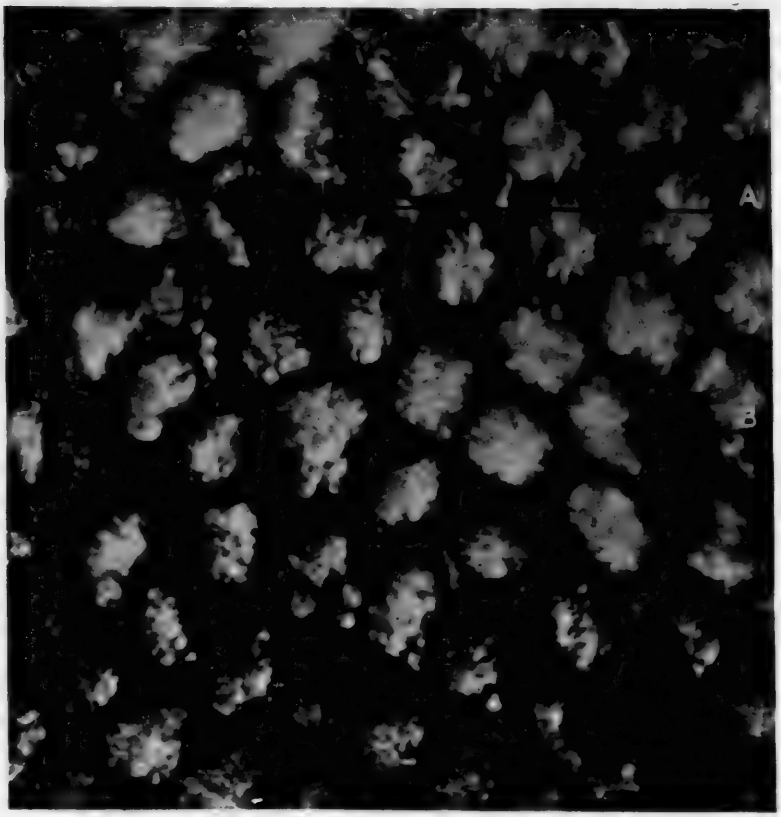

FIG. 17. This specimen, magnified about 2500 diameters, very clearly shows many of the large canals, *A* and *B*, situated in the cement-substance between the enamel rods. Each rod of defective tissue is also surrounded by an area stained black by the nitrate of silver solution.

enter an area in which the enamel is permeated with large canals, but we also everywhere catch glimpses of that sharply defined black line outlining the enamel rod. I wish to keep your minds fixed on the fact that this line is distinct from the large canals.

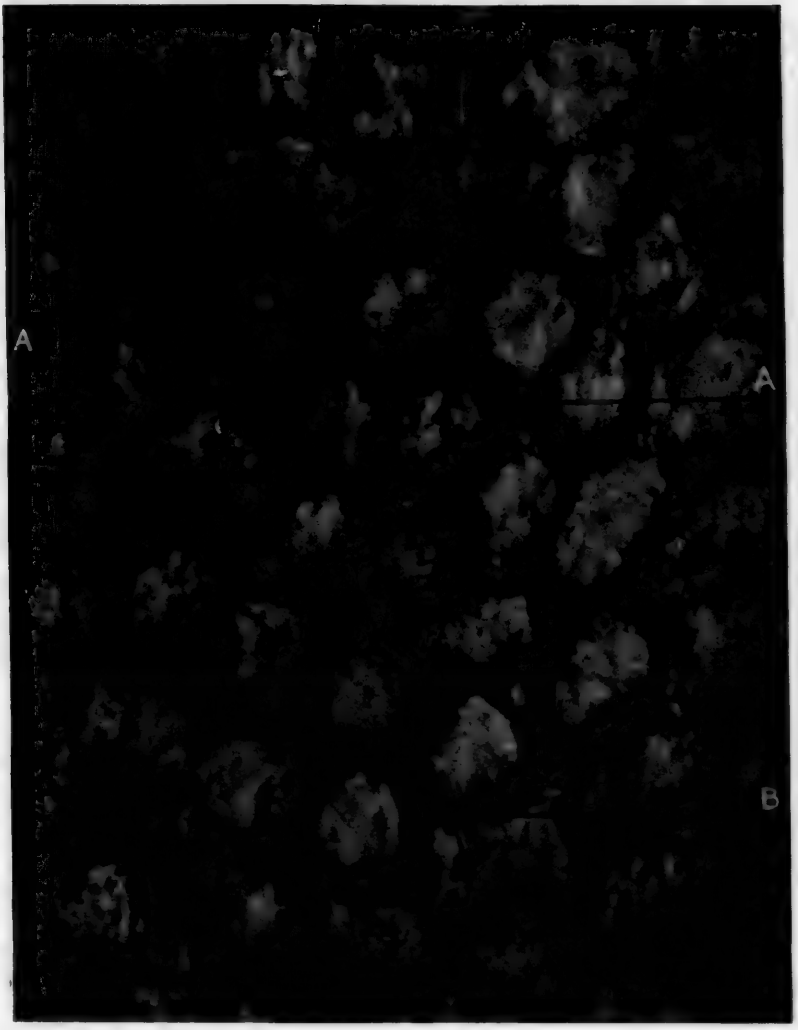

FIG. 18. Similar to *fig. 17*, but nearer to the area of sound tissue. Large canals are shown at *A* and defective rod sheaths at *B*. Magnified about 3000 diameters. The bridges or thornlike processes connecting the enamel rods are clearly shown in several places in this specimen.

It was Dr. Black's opinion that the defect in mottled enamel was largely or entirely confined to the substance between the rods. This opinion must have been based on an examination under low and medium powers of the microscope. With the high resolving power of the 2-mm. and 3-mm. apochromatic objectives we see that the enamel

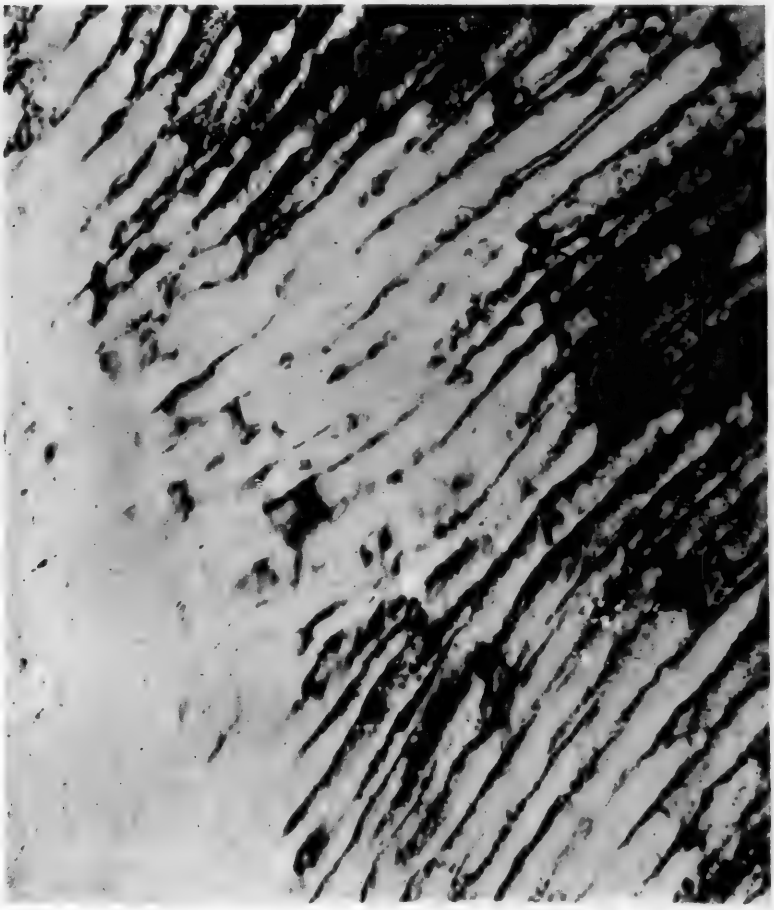

FIG. 19. Longitudinal view of mottled enamel. The lower part of the view is near the border line between sound and defective tissue. The upper part shows very defective calcification, which is most pronounced in the inter-rod substance.

rods are very imperfectly calcified. From my own point of view of the method of enamel calcification this is just what we should expect. Anyway, it is the fact. *Figs. 20, 21* and *22* show various aspects of imperfect calcification of the enamel rods as seen in longitudinal

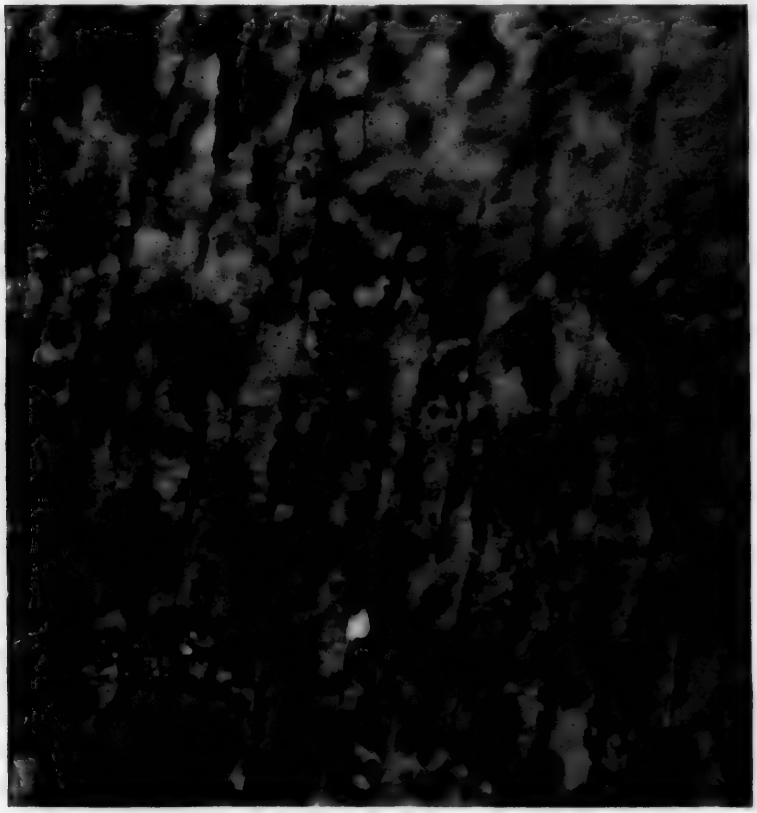

FIG. 20. In this specimen it is clearly seen that defective calcification in mottled enamel is not confined to the cement-substance between the rods as thought by Black. The substance of the rods is seen to be very imperfectly calcified.

section under high powers of the microscope. Imperfectly fused granules and small spherical bodies are everywhere in evidence in these specimens. Some of these larger globular masses appear to be identical with what have been hitherto described as "calcospher-

ites," but the appearances in these different specimens clearly indicate that the chemical changes in forming enamel and its molecular struc-

FIG. 21. Similar to *fig. 20*, but showing more clearly large defective spaces or canals between the rods.

ture are more complex and intricate than the inferences we might draw from previous descriptions of the formation of this tissue.

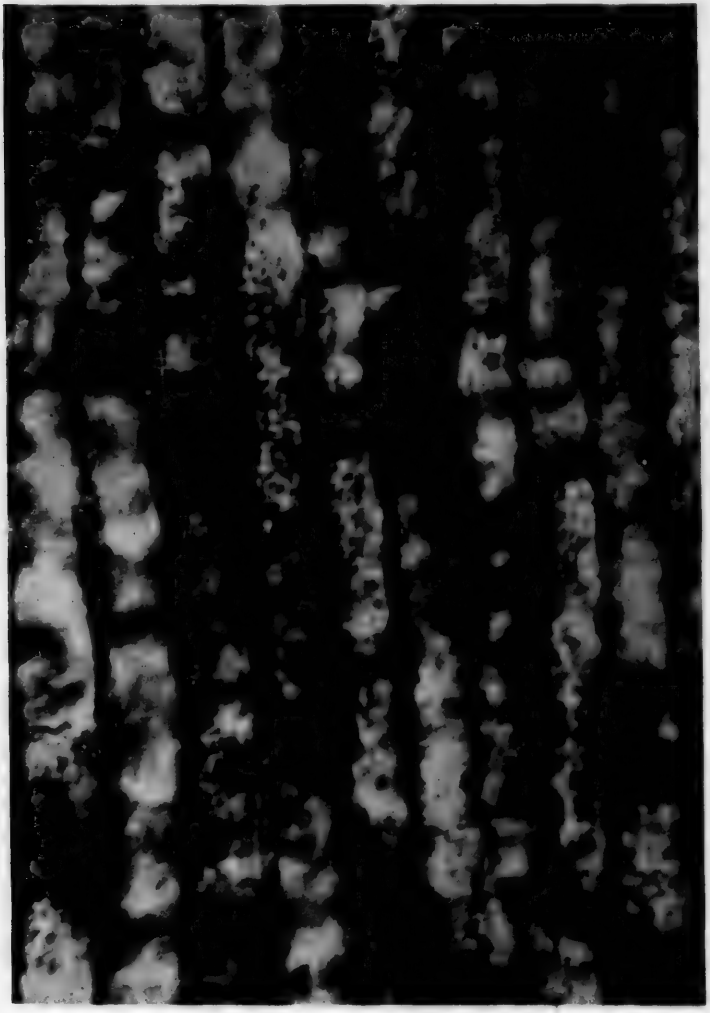

FIG. 22. Shows in several places in the rods the imperfectly calcified calcospherites of which the rods are built up.

III. ENAMEL OF FULLY FORMED BUT UNERUPTED TEETH

As the condition of mottled enamel is due to imperfect calcification, it occurred to me that a microscopic examination of the nearly completed but unerupted crowns of teeth might throw some light on the main theme. I was fortunate in being able to secure the skull of a large monkey in which the first permanent molars were in place, and the crowns of the upper centrals and some of the other teeth were nearly ready for eruption. Here we should expect to find the formation of enamel nearly finished but the processes of calcification or solidification not quite completed. Several of these teeth were removed from their crypts and treated with nitrate of silver solution for varying lengths of time precisely as were the specimens of mottled enamel. It was noticeable at once that these teeth, before being placed in the stain, had the paper-white appearance so characteristic of mottled enamel. And, as we shall see, the reason for this appearance is the same—the enamel was not completely calcified or solidified. In a transverse section of a molar (*fig. 23*) it is seen that the stain penetrated the entire thickness of the enamel in less than two hours.

Fig. 24 is from a longitudinal section of an upper central incisor. The formation of the crown of the tooth is nearly completed. The dentine and about half the thickness of the enamel were quickly stained with silver nitrate solution. On the buccal surface, inside the stained area, we have a fine view of the Schreger bands, which are due to the spiral arrangement of the enamel rods. In *fig. 25* we see a portion of *fig. 24* more highly magnified, in which the nature of the Schreger bands is still more plainly shown. As the significance and importance of this feature of enamel formation have been almost completely overlooked, I shall have more to say about it presently. *Figs. 26*, *27* and *28* show varying aspects of stained enamel, from unerupted teeth, in longitudinal sections. The appearances in some of these specimens so closely resemble mottled enamel that it would be quite impossible for me to distinguish between them. Here, as in the imperfectly calcified tissue of mottled enamel, it is the exterior portion of the enamel rod which first takes the stain. This is beautifully shown at the border line of the stain as seen under high powers. Such an area is shown in the small circle in *fig. 29*. Let us look at

some portions of this area under greater magnification (*figs. 30* and *31*).

If I had not previously seen these views and some one were to ask me the question, "Is that a view of mottled enamel or incompletely

FIG. 23. From the molar tooth of a monkey just before eruption. The nitrate of silver stain penetrates the full thickness of the enamel in a few minutes.

calcified forming enamel, or enamel in the early stages of decay," I have to confess that I should be unable to answer the query. The reason for this will, I hope, be made plain as we proceed. You will remember that I said, at the outset, that for me the chief interest of this investigation is found in the relationship shown between what

would seem, on first thought, totally unrelated conditions, and also in the light which these facts throw on the method of enamel formation.

FIG. 24. From incisor of monkey just before eruption of the tooth. The outer portion of enamel is not yet completely calcified and closely resembles mottled enamel in its appearance after staining. In the inner, unstained part of the enamel the Schreger bands are finely shown.

The sharply defined line surrounding the enamel rod, which the first effect of the stain shows, is seen in *fig. 32* and is the same as in mottled enamel. In some specimens, as in *fig. 32*, the rods are so

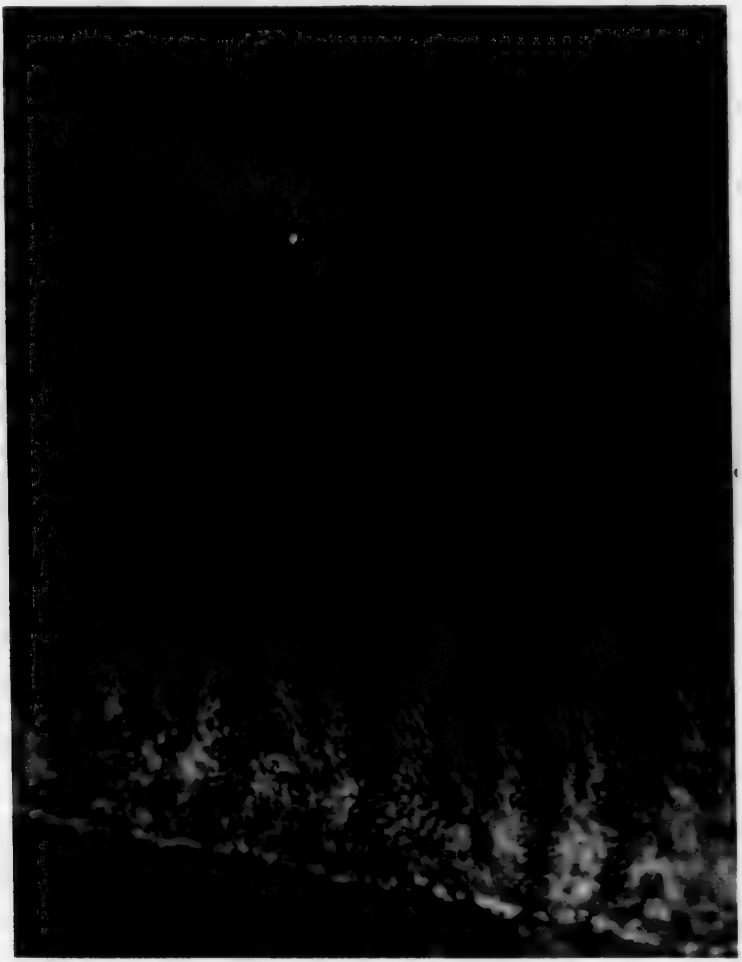

FIG. 25. A portion of *fig. 24*, under higher magnification, showing that the Schreger bands are due to the spiral arrangement of the enamel rods. In this view it is also clearly seen that the area around the enamel rods is first stained.

closely packed together that only the black outline is seen, there being but little cement-substance required to unite the rods. But the proof that this line is not what has heretofore been called the "interprismatic cement-substance" is found in the fact that in many of my speci-

FIG. 26. Another view from a completely formed but not completely calcified tooth (monkey). The stain quickly penetrates the areas in which calcification is not completed.

mens an unstained area is clearly seen between the rods *outside* this black line. This is finely shown in the longitudinal section of enamel under a power of about 3000 diameters, as seen in *fig. 33*. Here the border of each rod is shown sharply defined by a black line, but out-

side these black lines and between the rods there is an unstained area. This black line occupies the position of the limiting cell membrane of the ameloblast, but whatever interpretation is placed upon it, the fact must be recognized that it is a discrete substance.

FIG. 27. This photograph shows that when a stain enters the enamel through the dentine it always first penetrates through the center of the enamel whorls or spirals. (Molar of monkey.) The canals in these spirals will be shown in subsequent illustrations.

In *fig. 34* we have a view of incompletely calcified enamel cut transversely to the direction of the rods. This specimen was very lightly stained in section and then the section was ground away a little more. You see what has happened. On the margin, near the

surface, the granular, incompletely calcified substance has been torn away and loose particles of this substance may be seen. A portion of this view (*fig. 35*), under higher power, shows that these particles are small globular bodies of which the rods are built up, and these globular

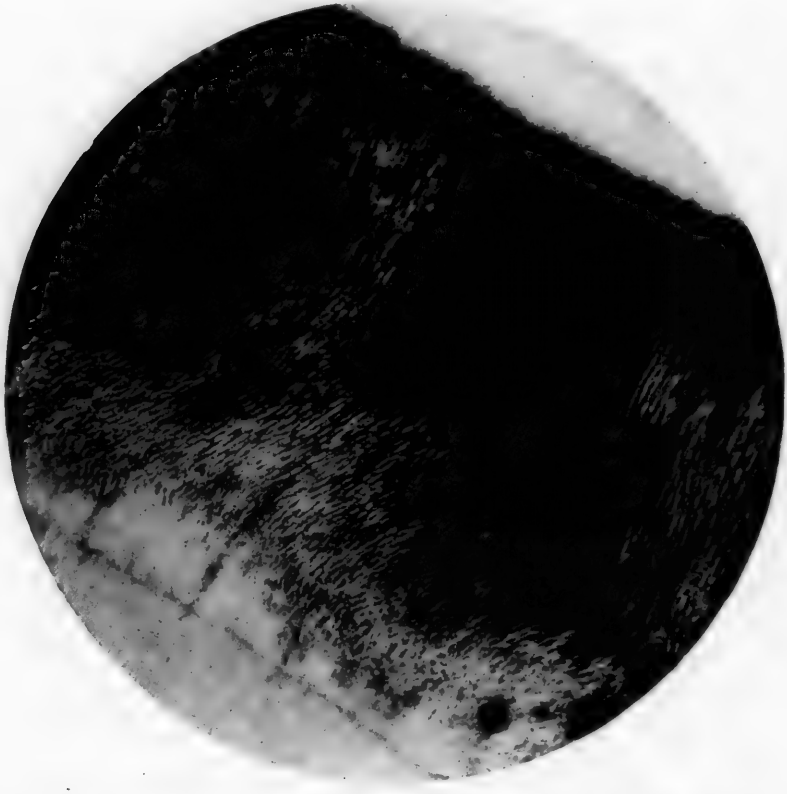

FIG. 28. Another stained specimen of young normal enamel in which calcification is not completed. (Molar of monkey.)

bodies were not yet completely fused into what would have been, a little later, the solid, perfectly formed enamel rod. These uncalcified, globular bodies are finely seen in *fig. 36*, which shows the rods in longitudinal section.

IV. ENAMEL OF MATURE TEETH IN NORMAL AND PATHOLOGICAL CONDITIONS

There are many aspects of this phase of my subject which I should like to dwell upon, but the limitations of time make it necessary to pass to the third and last section of my paper, which is devoted to the stained appearances of the early stages of caries in specimens of what would generally be regarded as normal human enamel. Let me emphasize the fact that nearly all of these specimens were selected from mouths showing exceptional freedom from decay, and in all instances, unless otherwise mentioned, the critical dentist would have passed these teeth as perfectly sound. That fact should be kept clearly in view in order to understand the full significance of what I shall show you.

Let us pass rapidly in review a few specimens of incisors, bicuspids, and molars which were first stained in bulk, and then sectioned and prepared for the microscope (*figs. 37, 38, 39, 40, 41,* and *42*). These photographs demonstrate two facts with striking clearness: (a) teeth specially selected for their apparent soundness and perfection are seen to have defective areas which the stain enters readily and rapidly; (b) the stain enters also the most perfectly formed enamel, although more slowly.

Let us now examine enamel in the early stages of caries. As I have previously pointed out, the necessary procedure in preparing balsam-mounted specimens for the microscope nearly always destroys or removes the bacterial plaque, but at the same time it reveals certain structural features of the enamel more clearly than can be seen in water- or glycerine-mounted specimens. *Fig. 43* shows a specimen in which the acid of decay has penetrated the entire thickness of the enamel. The stain has entered this area so completely that nearly all detail of structure is concealed. One of the most interesting phases of this early stage of caries is the reaction and change which we nearly always find in the dentinal tubuli *before there is any complete breaking-down of the enamel.* The nature of this change in the dentine and the reason for it are not well understood. *Fig. 44* shows another specimen of the early stages of caries under a somewhat higher magnifying power. *Fig. 45* shows finely the passage of the stain down channels or

FIG. 29

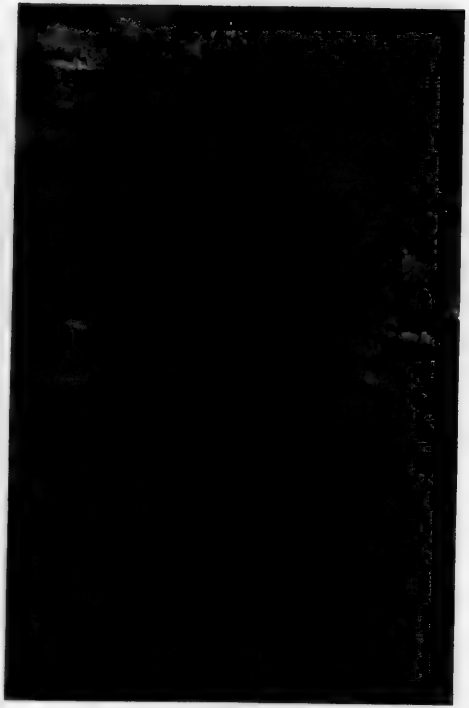

FIG. 30

FIG. 29. Here, as in mottled enamel, our most instructive views are found on the border line of calcification. The two following illustrations are from that part of *fig. 29* shown in the small circle. (Molar of monkey.)

FIG. 30. The appearance here so closely resembles the earliest stage of caries of enamel that it is impossible to define the one from the other. (See *fig. 29*.)

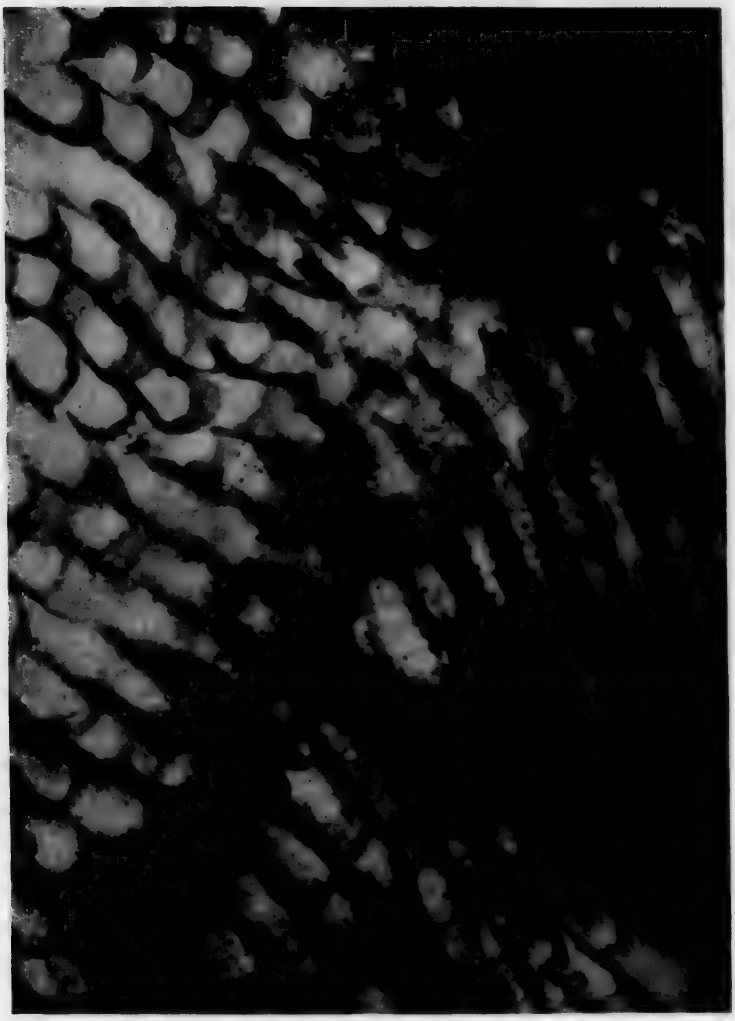

FIG. 31. This view closely resembles mottled enamel and well illustrates the intimate relation between incompletely calcified enamel, mottled enamel, and the early stages of decay. (See *fig. 29.*)

canals which have been enlarged by the processes of caries. *Fig. 46* is a deeply stained specimen which shows very clearly the border

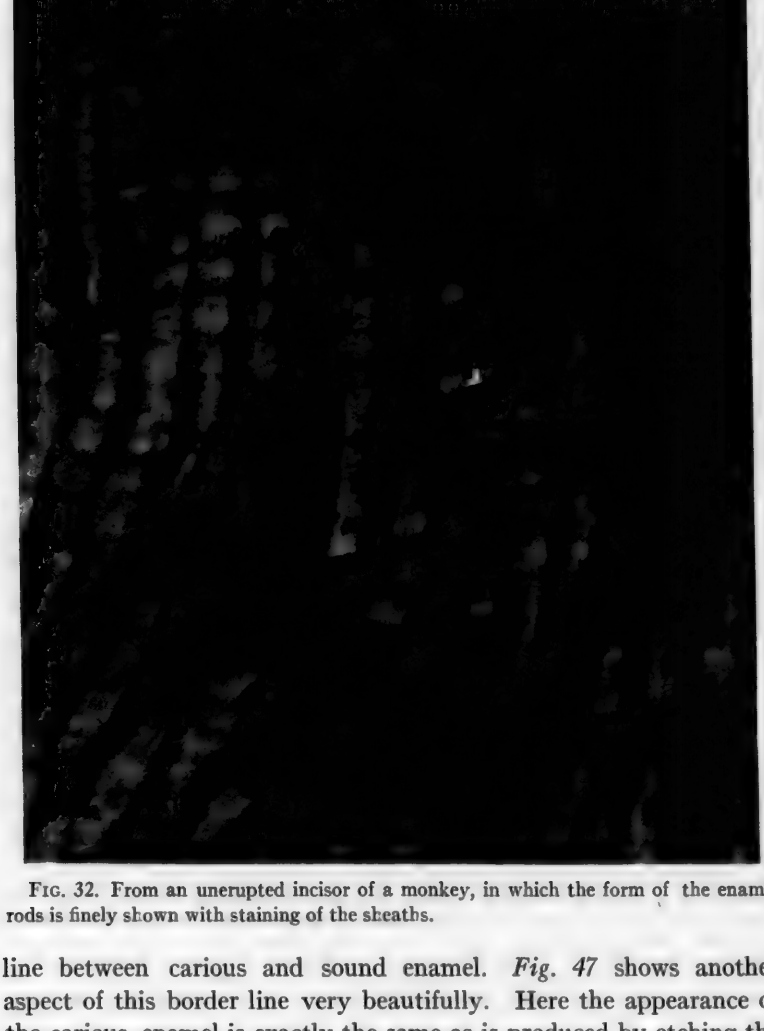

FIG. 32. From an unerupted incisor of a monkey, in which the form of the enamel rods is finely shown with staining of the sheaths.

line between carious and sound enamel. *Fig. 47* shows another aspect of this border line very beautifully. Here the appearance of the carious enamel is exactly the same as is produced by etching the

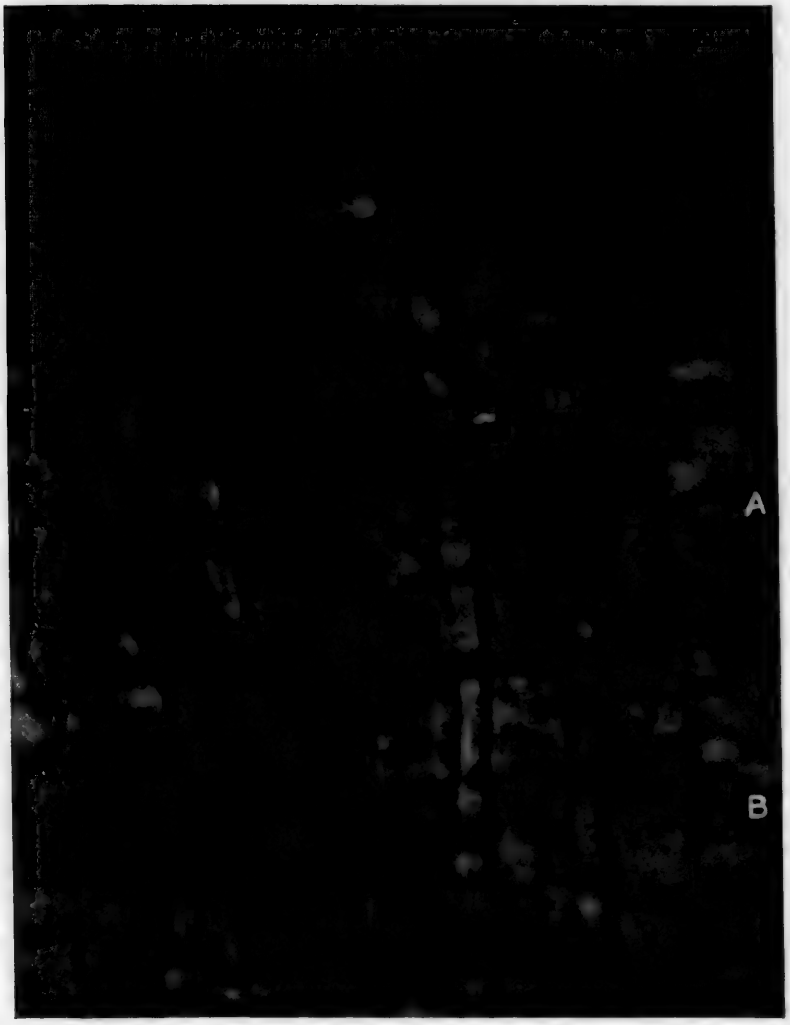

FIG. 33. Enamel rods from molar of monkey, magnified about 2000 diameters. Here the sheaths of the enamel rods in longitudinal section are finely shown deeply stained. Between the rods, *outside the sheaths*, considerable cement-substance is seen, as at *A* and *B*.

surface of a section with lactic acid. *Fig. 48* is a general view from a lightly stained specimen photographed so as to show all detail of early stages of caries of enamel and the relation of the carious to the

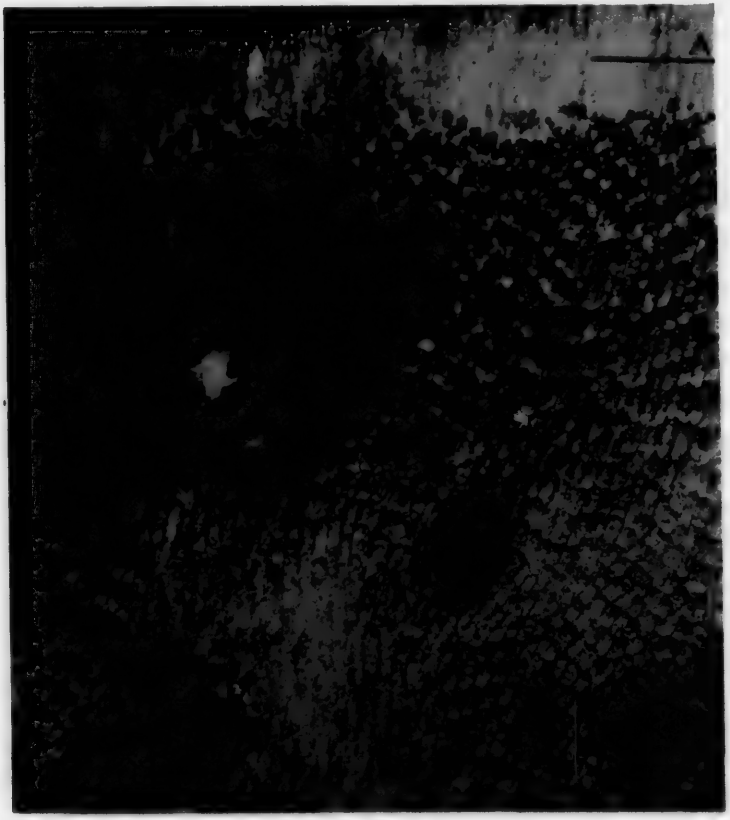

FIG. 34. From a fully formed monkey incisor just before eruption. Calcification is not complete. The specimen was ground obliquely across the enamel rods. The calcospherites of which the sections or varicosities of the rods are built up are not well consolidated, and are torn apart in the process of grinding. At *A* all of the calcific material has been torn away, leaving only the rod sheath or organic matrix. (See *fig. 35*.)

sound tissue. In *fig. 49* we have a portion of *fig. 48* more highly magnified, and with the detail of the canals of decay more sharply contrasted.

We pass to an examination of a few transverse specimens of carious enamel under magnifying powers of from 600 to 2000 diameters. In these views the whole story of the carious process is told as far as the

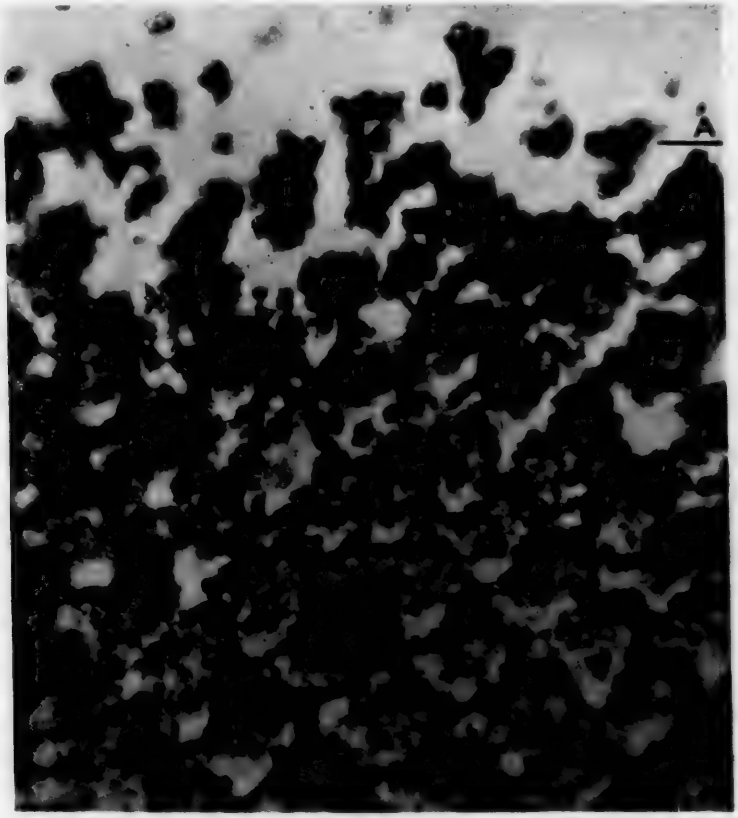

FIG. 35. This is a small portion of *fig. 34*, near the margin shown at *A*, under higher magnifying power. The incompletely consolidated calcospherites, as torn apart by the grinding of the section, are plainly seen. All of the specimens of this character were prepared by slow saturation in Canada balsam, which was then hardened by low heat in a special chamber.

mechanical aspects are concerned. In *fig. 50* we begin, at the top, with the very commencement of the carious process. In most instances it is not a round or irregularly-shaped canal that we find as

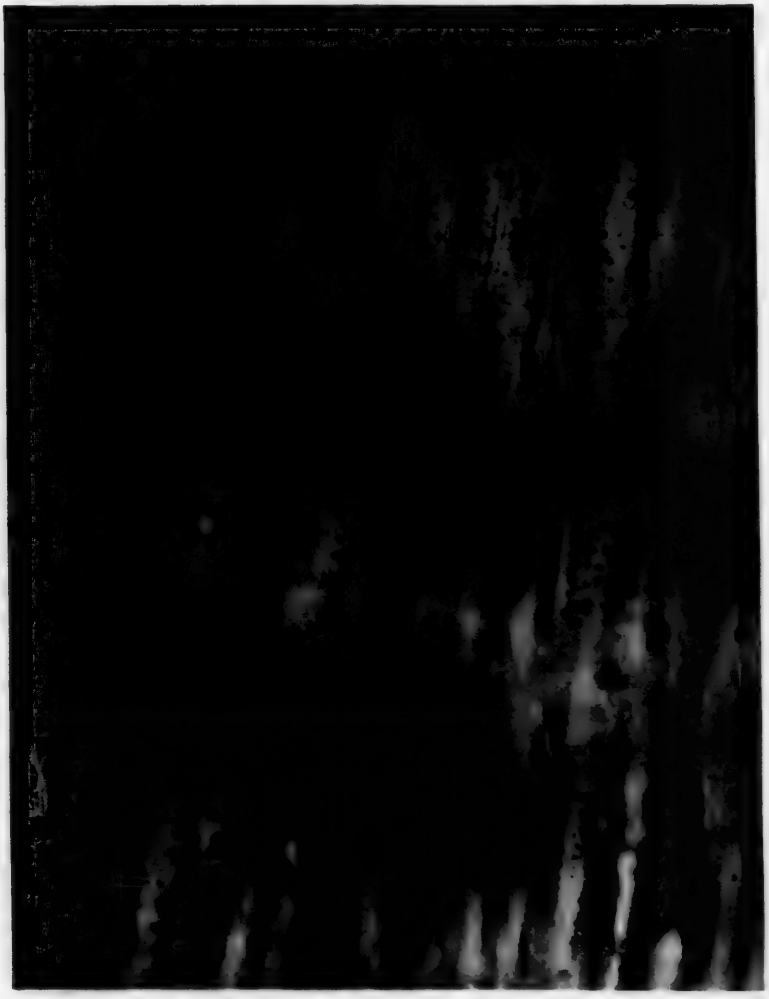

FIG. 36. A longitudinal section from material similar to that shown in *figs. 34* and *35*. Deeply stained calcospherites of varying sizes are plainly seen throughout the rods in this illustration.

representing the initial action of decay, but the stained area is, as I have said, a sharply defined area corresponding with the circumference, or a part of the circumference, of the enamel rod. Except where

FIG. 37. From a section of an upper molar human tooth which appeared to be quite sound. The nitrate of silver stain has revealed the commencement of decay on the proximal surfaces and other imperfections.

the rods are packed very closely together, there is an unstained area between the rods outside this sharply defined line. This is a new fact in the histology of enamel and shows that the outside of the enamel rod is bounded by a discrete or differentiated substance. The forma-

FIGS. 38–42. *Figs. 38, 39, 40, 41* and *42* represent sections from human teeth specially selected as among the best that could be found in a large collection. The stain readily penetrates the enamel in all of these and reveals various defects.

FIGS. 38–42. *Figs. 38, 39, 40, 41* and *42* represent sections from human teeth specially selected as among the best that could be found in a large collection. The stain readily penetrates the enamel in all of these and reveals various defects.

tion of enamel is not quite the simple process that some dental histologists would have us believe. There is very much more to be learned about it. As we pass toward the lower part of the picture, we observe

FIG. 43. This new series, showing early stages of caries of enamel, is introduced here for reasons mentioned in the text. This illustration shows that the process of decay has completely penetrated the enamel and produced a marked reaction in the dentine before there was any appearance of an external cavity. The stain has completely obliterated all detail in the enamel. To show this detail we must stain more lightly, as shown in some of the following illustrations.

these black, crescent-shaped spots becoming larger and larger as the processes of decay melt down the walls of the channels, until, near the bottom of the view, the channels have nearly coalesced and the enamel

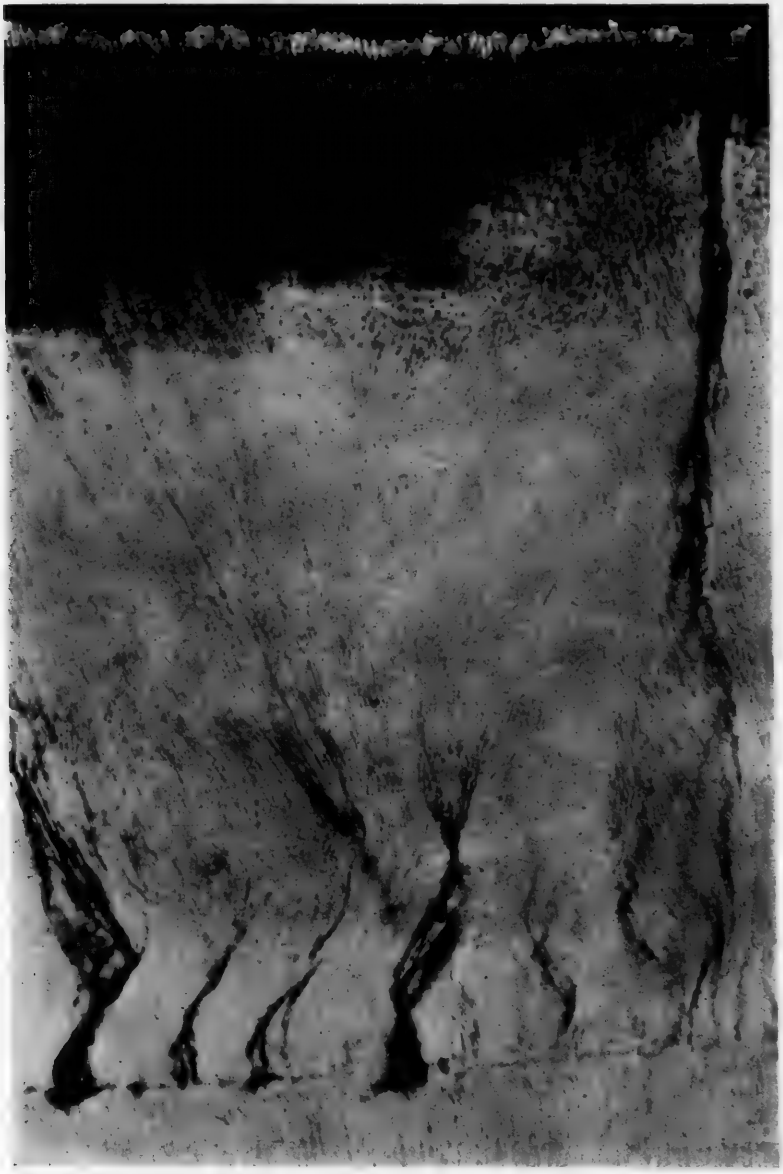

FIG. 44. Shows the earliest stage of enamel decay. The section is cut slightly obliquely to the long axis of the enamel rods so as to show the canals produced by the bacterial products of the plaque, which is here removed.

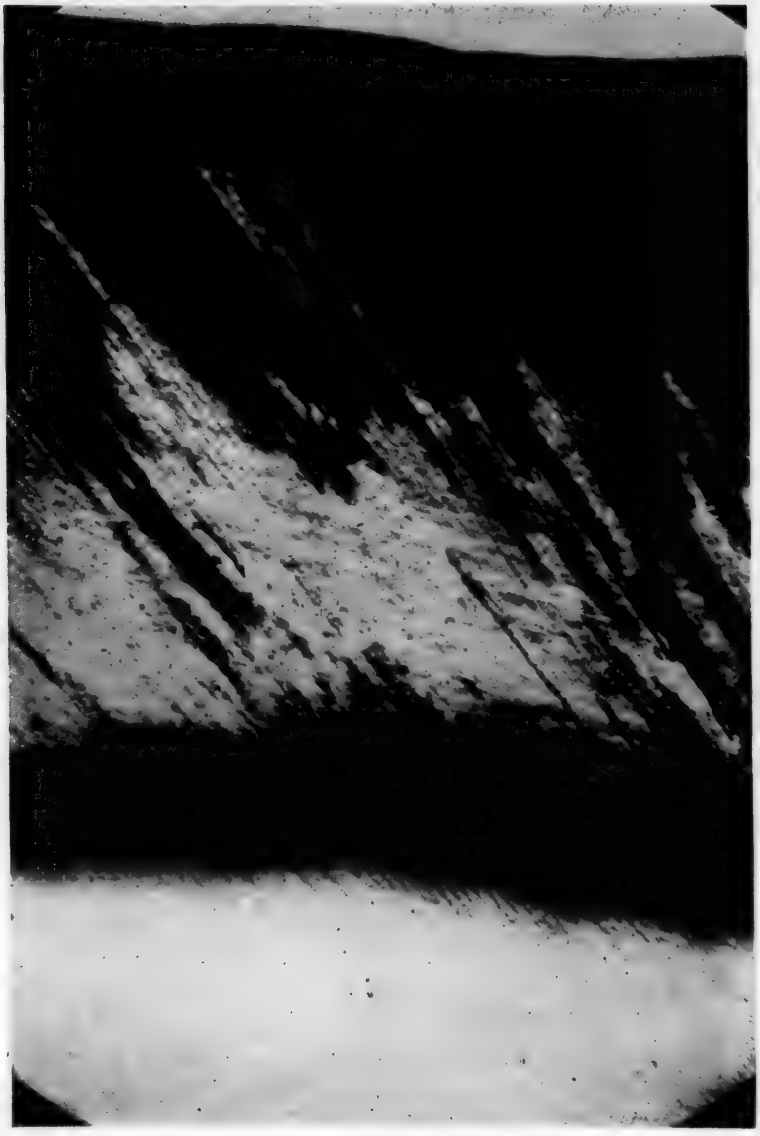

FIG. 45. From a longitudinal section of the early stages of caries of enamel. Here the stain is seen flowing down canals which have been made by the products of bacterial action.

is just on the point of breaking down into a cavity. *Figs. 51* and *52* show the process under higher power and more clearly. *Fig. 53* is from a section cut obliquely across the enamel rods and shows the

FIG. 46. An overstained but deeply interesting specimen as showing very clearly the dividing line at *A* between carious and sound enamel. The enlarging of the space between the enamel rods, giving them a pointed appearance, is one of the characteristic features of enamel decay. The large canals seen here show that decay has made considerable progress.

earliest stage of caries. *Fig. 54* is from a specimen cut from a molar that was apparently perfectly sound. It shows the stained outline of the rods with the sharpness of a drawing.

What is the nature of the space on the circumference of the enamel

rod which is represented by this black line? Keeping in mind the fact that in the finest specimens of enamel that we can find the periphery of the rods shows a sharp reaction to stain before the substance

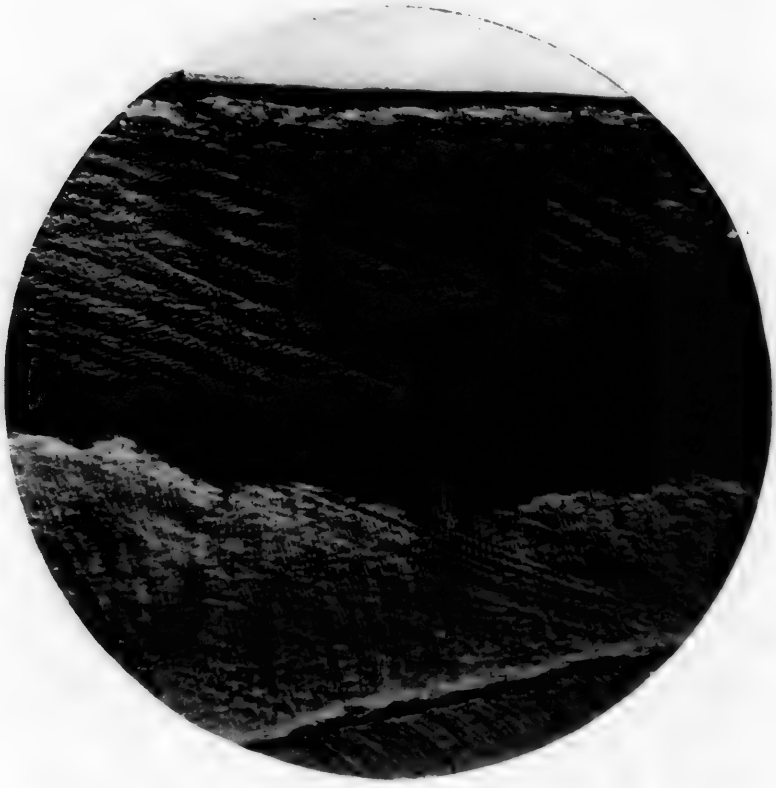

FIG. 47. A fine illustration showing the change produced in enamel as seen under a power of about 200 diameters. We shall find that the appearances seen here, under low powers of the microscope, show that the structure of enamel in the early stages of decay closely resembles that found in mottled enamel.

of the rod begins to stain, what may we conclude about it? I think that we may assume that this line represents a distinct discrete substance different from the enamel rod or the cement-substance which lies outside this line. It is much more selective to stain than the other

parts of the enamel. In mottled enamel this outer part of the enamel rod is imperfectly formed. It is evidently in a soft or cartilaginous condition, and in dried specimens is largely a space filled with air. The conclusive proof of this is seen in *fig. 55*. This is an unstained specimen of mottled enamel from a dried tooth in which nearly the entire thickness of the enamel was defective. When this specimen was first mounted, all the defective area was dark by transmitted light, like the spot shown at *A*. While I was studying it through the microscope, the dark area began to clear up *with the formation of air bubbles*. By working rapidly I was able to secure a good photograph before the clearing process was completed.

Figure 56 is an exceedingly interesting specimen. It represents a section of mottled enamel from a tooth, lightly stained in bulk with nitrate of silver, which was first ground to a thickness of about 1 mm., polished on one side and cemented to a glass slide with Canada balsam. After this had hardened, the section was ground to a thinness of about 5 microns and treated with a 3 per cent solution of lactic acid until the enamel was nearly decalcified. Something very important is revealed at *A*, which represents the surface of the enamel. A very distinct line is seen here, which occupies the position of Nasmyth's membrane. In mounting, pressure on the cover glass has forced the membrane away from the surface of the enamel. But the arresting thing to which I especially call your attention is those lines which pass from the membrane to which they are attached down *between* the enamel rods. These lines undoubtedly represent a cross section of the sheaths of the enamel rods to which I have already called your attention. Some of these lines have been destroyed in the process of grinding, but all of those that remain can, in the original photograph, be clearly traced as passing between the rods.[2]

We have here evidence that seems to me very nearly conclusive that the periphery of the rod is the last part to be calcified. The appearance is as if the energies governing calcification became somewhat exhausted toward the finish of this process and were not quite able to supply sufficient calcific material for filling in the inter-rod spaces. This view finds confirmation in the fact that the outer portion of the enamel is nearly always the most defective in this respect.

[2] See the Addendum, page 190.

In nearly all cases of mottled enamel it appears that something happened quite suddenly, when the process of calcification was partly completed, which prevented the normal physiological processes concerned with calcification from functioning properly. To find out

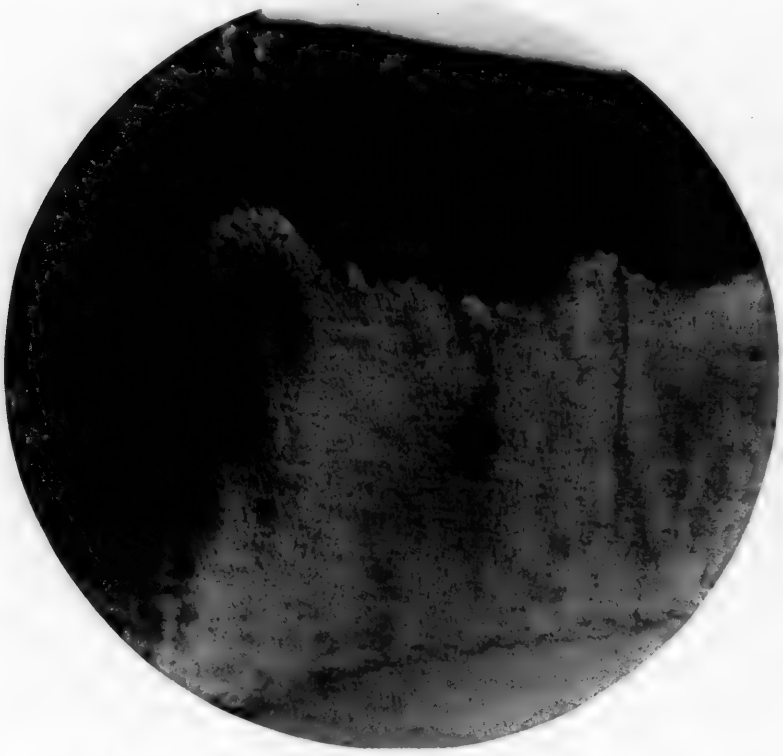

FIG. 48. A general view from a lightly stained specimen photographed so as to show all detail of early stages of caries of enamel and the relation of the carious to the sound tissue. (See *fig. 49.*)

just what happens at this stage will, I fear, prove a somewhat baffling problem.

In caries of enamel the tearing-down process is the reverse of the building-up process. In enamel formation calcification ends with the

completion of the inter-rod substance. The beginning of the carious process is in the dissolution of this inter-rod substance. This last-formed material is less resistant to the acid of decay than the substance

FIG. 49. A portion of *fig. 48* more highly magnified and with the detail of the canals of decay more sharply brought out.

of the enamel rods. If, as I contend, the enamel rod represents the calcified cytoplasm of the ameloblast, this is exactly what we should expect, but it is to be clearly understood that this want of resistance

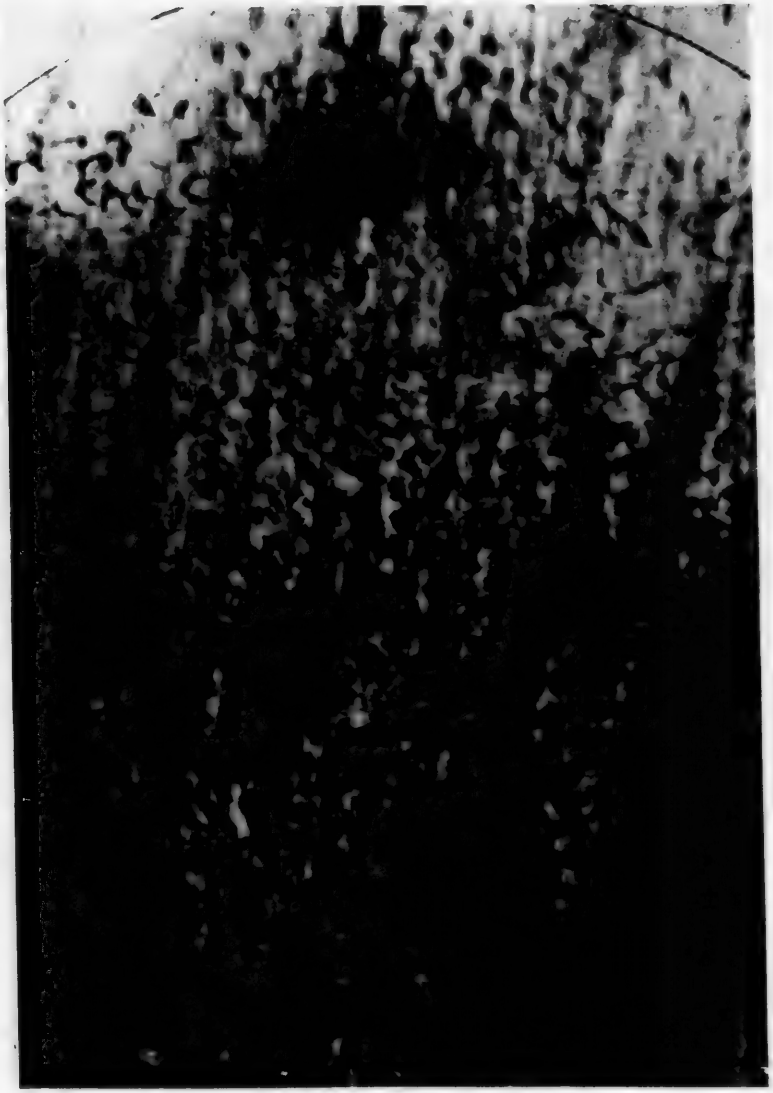

FIG. 50. Transverse section of enamel showing the progress of caries from the earliest stages, as seen in the upper part of the view, to the point where the enamel is nearly on the point of breaking down into a cavity, as seen in the lower part of the illustration. Obj. 3, proj. oc. 6. (See *fig. 51.*)

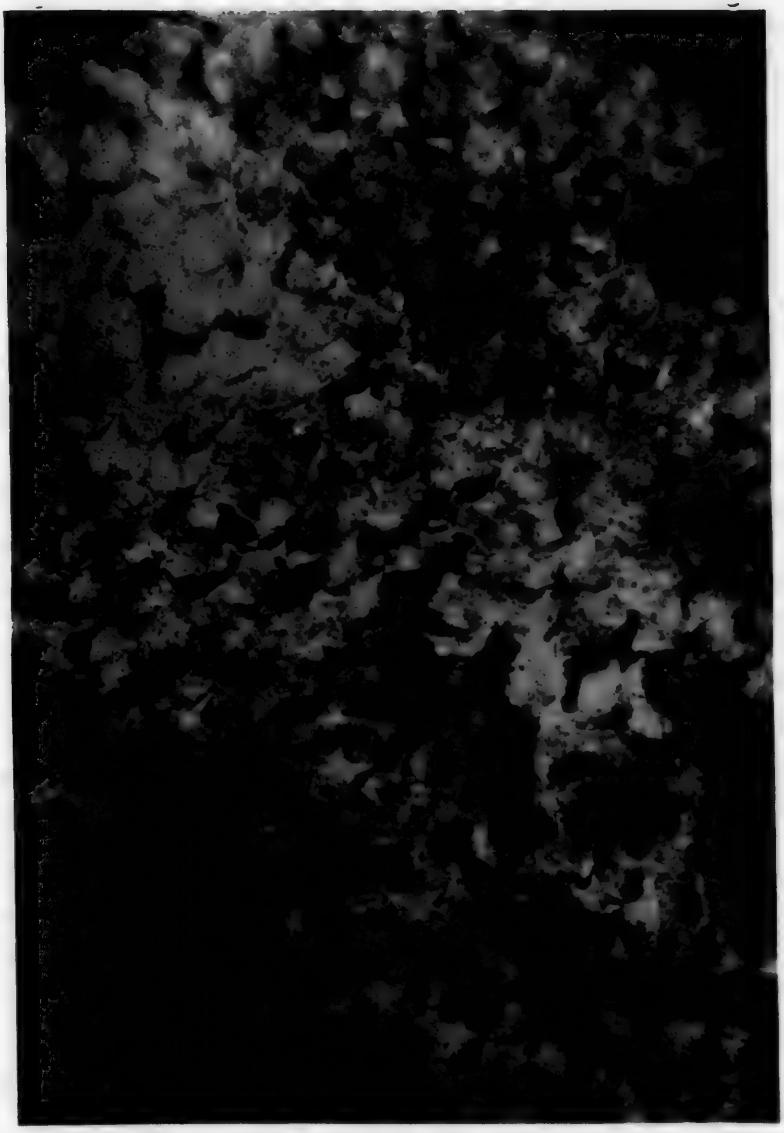

FIG. 51. From a specimen similar to *fig. 50*, but more highly magnified. Comparatively slow decay. The progress of decay is strikingly shown from the upper to the lower part of the illustration. It is seen to begin with an enlargement of the space occupied by the sheath of the enamel rod. This continues to enlarge and encroaches more and more on the substance of the enamel rod until the canals merge into each other and the enamel breaks down. (*See fig. 52.*)

FIG. 52. Similar to *fig. 51*, but showing more rapid decay. As far as the physical appearances of the enamel itself are concerned, the whole story of the effect of the processes of decay is here perfectly revealed for the first time.

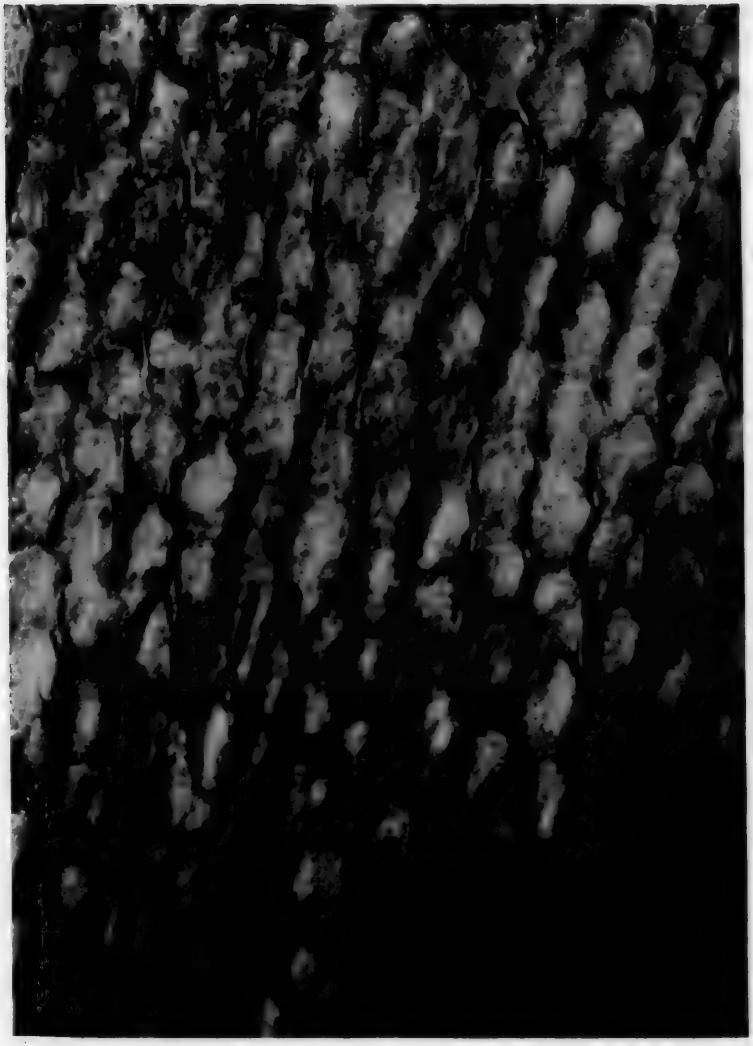

FIG. 53. From a section ground obliquely across the enamel rods and showing one aspect of the very earliest stages of caries of enamel. This early stage is marked by the appearance of small canals and rapid staining of the sheaths of the enamel rod. It is possible that some structural or chemical defect in this sheath predisposes the enamel to decay. But the active agents of decay are obviously bacterial products. Note the striking resemblance between this and *fig. 31*.

does not apply to the sheath of the enamel rod; *that* is more resistant to acid than any part of the enamel except Nasmyth's membrane.

There is one very important phase of this subject to which I wish

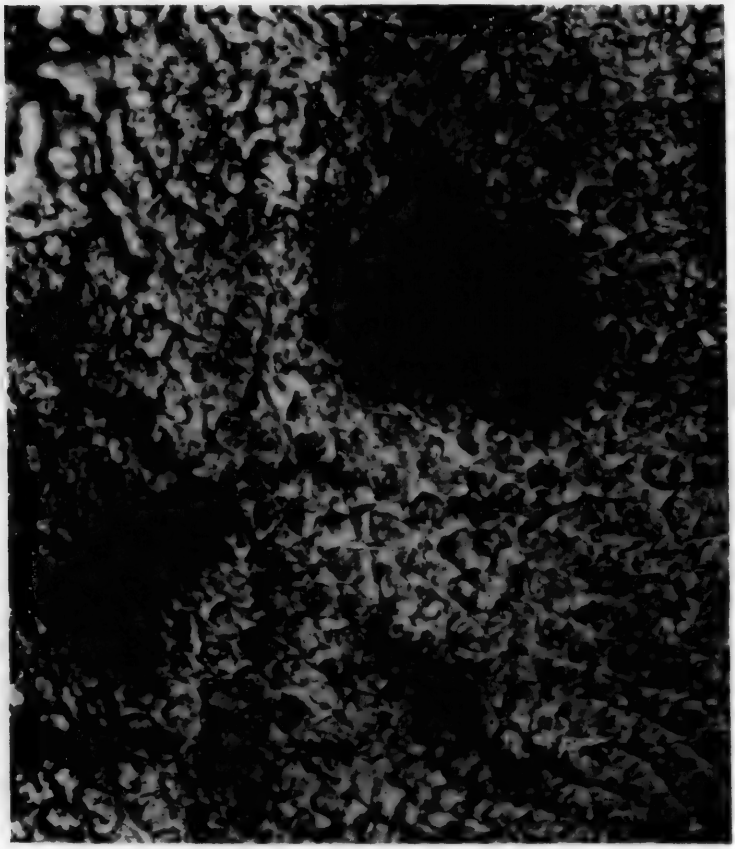

FIG. 54. This illustration shows various phases of imperfections in what appeared to be a perfectly formed tooth. There was no appearance of caries on the surface of the enamel, but it is easily seen that if decay were to attack such a tooth it would proceed more rapidly than in one of better structure.

especially to direct your attention. When examining the unerupted teeth of the monkey, I called your notice to the so-called enamel "whorls." These whorls have been looked upon as imperfections in

enamel formation. I regard this as one of the most serious mistakes that have been made in the histology of enamel. These enamel whorls represent the spiral formation which is fundamental to all the

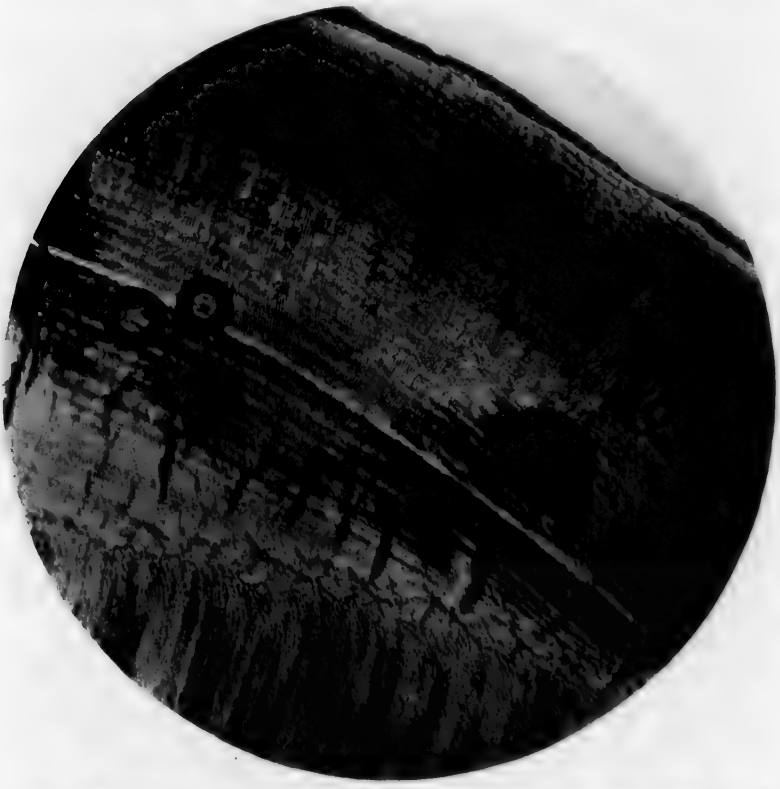

FIG. 55. Showing that enamel areas that appear dark by transmitted light may be due, in dried specimens, to imprisoned air. When this specimen was first mounted, nearly all of the enamel was dark like the spot shown at the right. It soon began to clear, with the formation of large bubbles as shown. The dark appearance might often lead to a misinterpretation of structure.

best types of enamel. These spiral arrangements of the enamel rods are found in greatest abundance in teeth which have to bear the greatest stress, as in the molars and bicuspids. They are also more abundant on the lingual surface of the upper incisors than on their labial

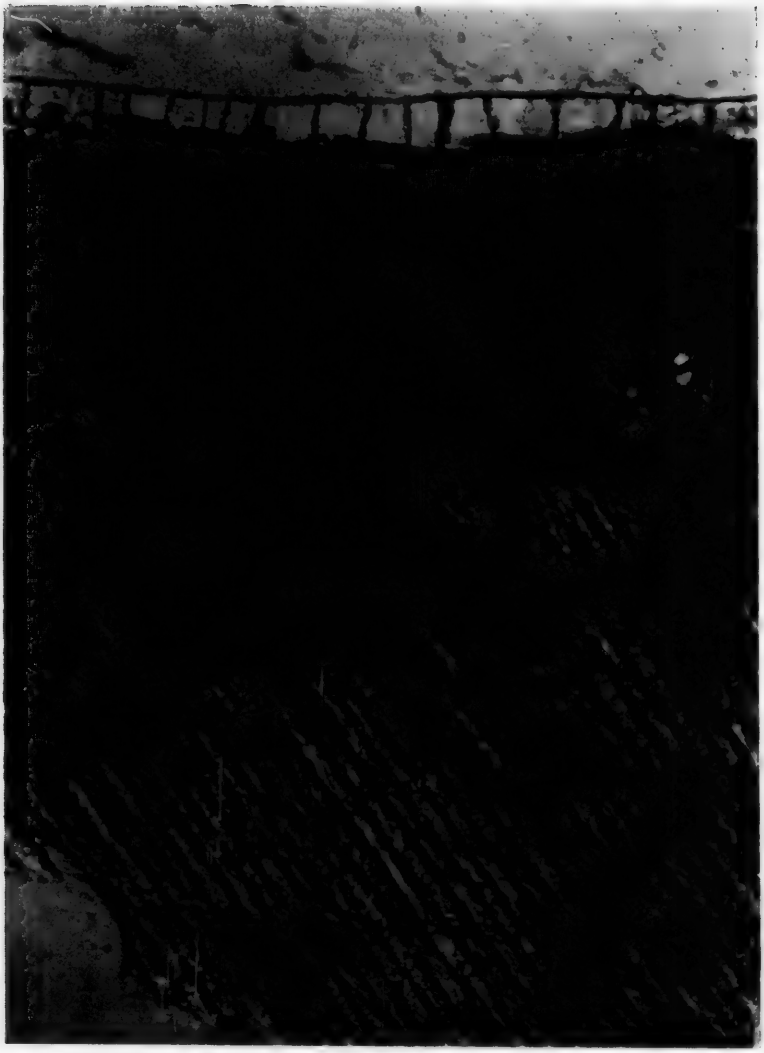

FIG. 56. From longitudinal section of enamel from human tooth magnified about 600 diameters. The enamel was partially decalcified with lactic acid. Nasmyth's membrane is seen forced away from the surface of the enamel by pressure on the cover glass. Passing from Nasmyth's membrane, and attached to it, are seen longitudinal sections of the sheath of the enamel rod. Many of the thornlike processes which unite the rods are also seen in this photograph.

surface. I have made a rough calculation, based upon counting the numbers in transverse and longitudinal sections, and I conclude that there are from 8000 to 10,000 of these spirals in a molar tooth. In *fig. 57* about 200 of these whorls may be counted in a single thin section. An enlarged portion of *fig. 57*, seen in *fig. 58*, shows these spirals more clearly. These spirals are much more complicated in the powerful gnawing teeth of the beaver than in any others which I have examined. Now, the center of these spirals is tubular. When staining fluids enter the enamel by way of the dentine, the stain always enters first the center of each spiral. This is very plainly seen in *figs. 59* and *60*. When the stain enters from the surface of the enamel, it passes through the thickness of this tissue and enters the dentine by way of the tubes in the center of the enamel spirals, as shown in *figs. 61* and *62*. In transverse sections of the spirals these tubes or canals are very clearly shown (*figs. 63* and *64*).

We have now demonstrated that fluids may enter the enamel by way of the tooth pulp and the dentine, and also from the surface. The way is open for calcific material to enter the enamel by way of the dentine and to be deposited there, thus increasing the solidity of the enamel, if such process is possible under the conditions governing constructive metabolism in the human body. Have we any evidence that such metabolism occurs? None whatever, as far as I can see at present.

Just consider for a moment the condition we have in mottled enamel —an open, loosely constructed condition of this tissue. If a building-up and consolidation of enamel could take place by means of calcific material brought into it by way of the dentine, here is the most striking condition in which such phenomena might be exhibited. We might say that here nature has a great opportunity for showing what she can do in the way of repairing defective structure. But when mottled enamel from the mouths of people fifty or sixty years of age is examined, do we find any evidence that forty years' functioning of the dental pulp has improved the structure of the enamel? Not the least, as far as I know. The teeth present the same opaque white appearance at sixty as they do at sixteen. Stain enters the enamel freely and rapidly in the older teeth as in the younger. But we saw that in unerupted teeth—and the same thing is true of recently erupted

teeth—stain enters the enamel much more rapidly than in older teeth. What is the explanation of this? My present view is that hardening processes may go on in enamel for a long time, perhaps for many years, *without the addition of any new material.* And there is nothing

FIG. 57. Showing the enamel whorls or spirals under low power. The stain is seen entering the enamel from the dentine through these whorls and also from the surface. (See *fig. 58.*)

surprising in this. Is it not exactly what occurs in all mineral cements? I think of the process as a *tightening of molecular grip.* Perhaps there is a slowing-down of the rate of molecular or atomic vibration. I greatly doubt if there can be any metabolic or physiologi-

FIG. 58. A part of *fig. 57*, enlarged, showing the character of the whorls or spirals. The stain always enters the center of the spirals and follows the spreading course of the rods.

cal action, any true nutrition, in any tissue in the absence of the cells which we believe govern or control all of these activities.[3]

My view of the method of enamel formation, a method which I think explains some of the new views of enamel structure we have

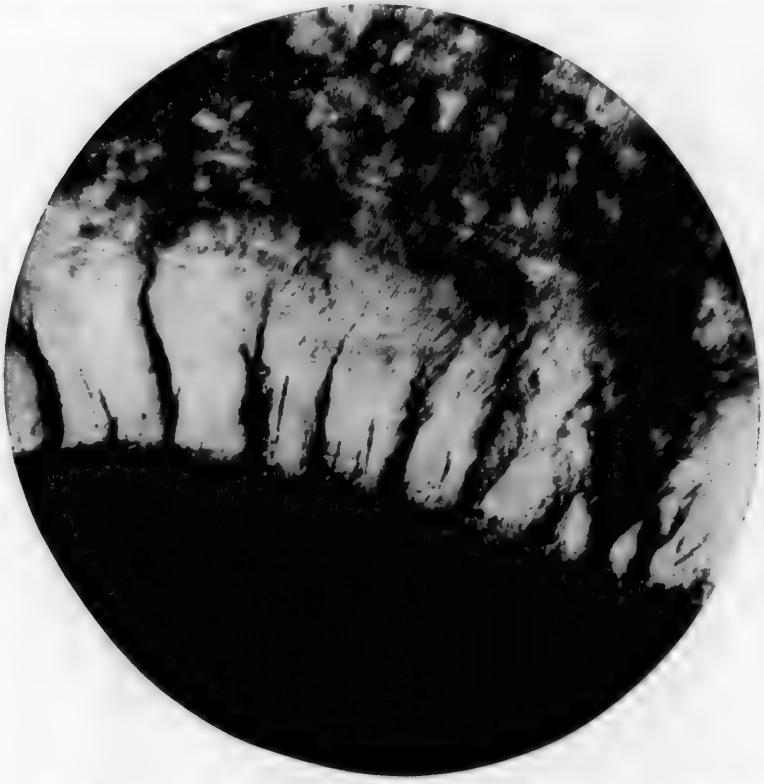

FIG. 59. Shows stain entering enamel from the dentine by way of the center of the enamel spirals and, following the course of the enamel rods, spreading out until the entire tissue becomes deeply stained. (See *fig. 60.*)

been examining, is that the enamel organ has two distinct functions. The function of the *ameloblasts* is to give form and character to the enamel rods. The enamel rods are the calcified ameloblasts. The

[3] See the Addendum, page 191.

stratum intermedium is the secreting part of the organ. In the rodents, fishes, and to a less degree in many other animals, the stratum intermedium shows all the characteristics of typical secreting structures. This part of the enamel organ selects the calcific material from the blood. This albuminlike material has been called "calcoglobulin." Calcification begins in small spherical masses which are called "calcospherites." They first appear near the lower end of the ameloblasts. The *calcific* material of the enamel rod and the inter-rod substance is

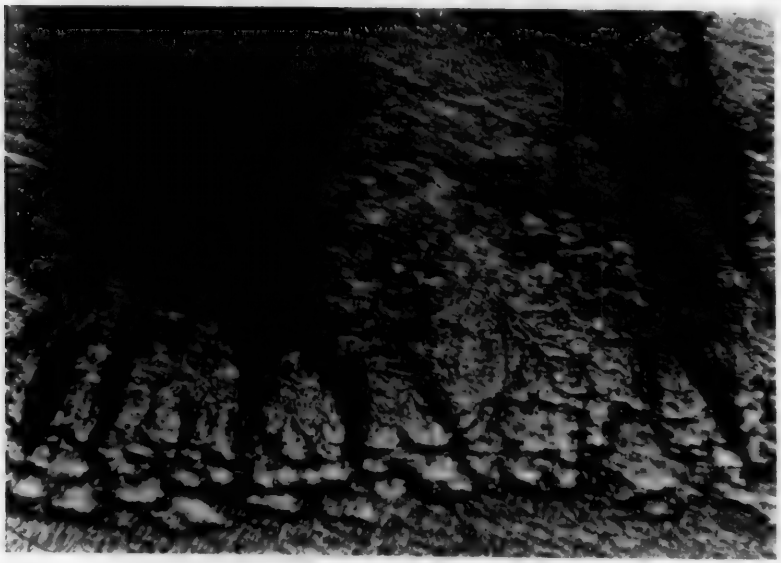

FIG. 60. Similar to *fig. 59*

identical. The difference in appearance in ground sections in enamel rod and inter-rod substance is due to the cytoplasmic structure of the ameloblast. This calcified, cytoplasmic substance of the enamel rod naturally offers more resistance to the process of decay than calcified material alone. Imperfect calcification is probably due to imperfect functioning of the secreting part of the enamel organ.

We are just beginning to learn something of the intimate relations of the secreting organs of the body, and it is highly probable that we shall sooner or later discover that mottled enamel and other similar

conditions of this tissue are due to a disturbance of balance in the functioning of the endocrine glands.

We are always primarily concerned with facts—they must have first consideration. And here are some of the important facts which

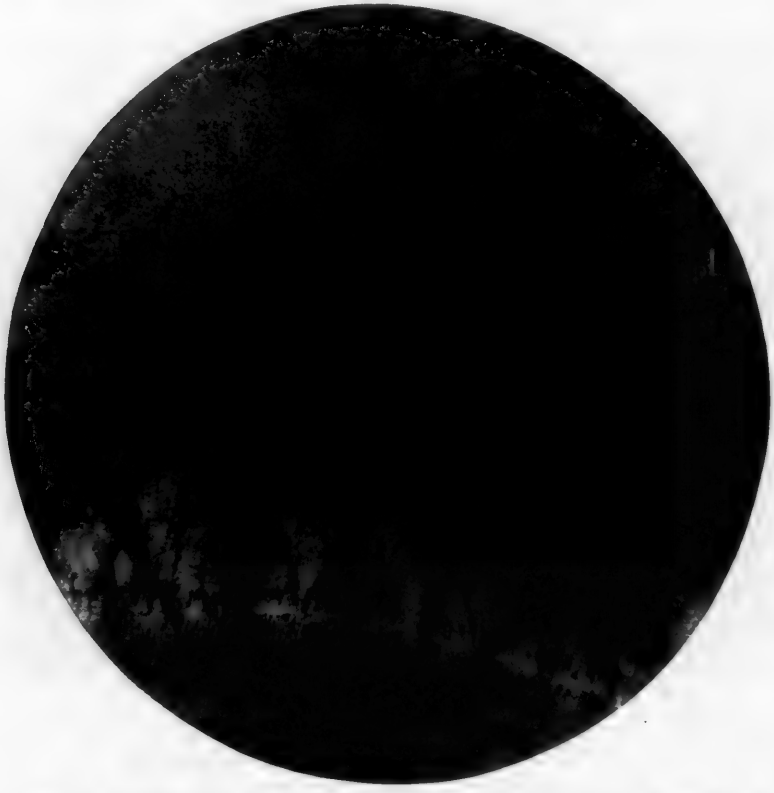

FIG. 61. Showing stain entering from the surface of the enamel. It follows the course of the enamel rods and enters the dentine through the center of the spirals. (See *fig. 62.*)

we have to face and explain in our efforts to solve the probem of dental caries. While we find a considerable variation in the permeability of the enamel of different teeth, we find that the enamel of all teeth is permeable to some extent. All enamel is most permeable on its sur-

face. Where a stain can enter the enamel we have no reason to suppose the acid of decay may not enter. We have shown in this paper how the acid of decay begins its work in caries of enamel by the enlargement of those natural canals which are found in the outer

FIG. 62. Similar to *fig. 61*. Note the large stained area in the dentine where the stain has flowed in from the enamel.

layers of all enamel. This process begins beneath bacterial plaques which contain microörganisms capable of converting starch and sugar into lactic acid. As long as these microörganisms remain active as acid-producers, fastened to the surface of the enamel, this process of the dissolution of the enamel is bound to go on. But this process of

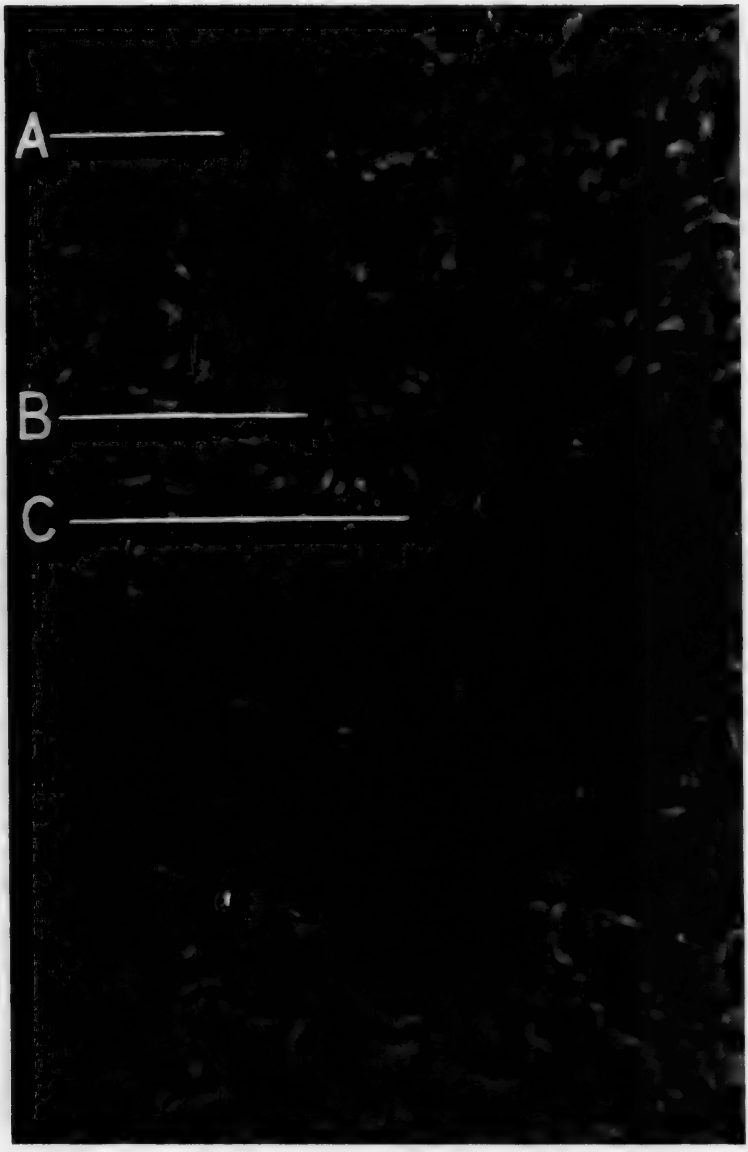

FIG. 63. Cross section through an enamel spiral magnified nearly 3000 diameters, showing the tubes or canals, at *A*, *B*, and *C*, through which the stain enters and is distributed throughout the enamel. (See *fig. 64*.)

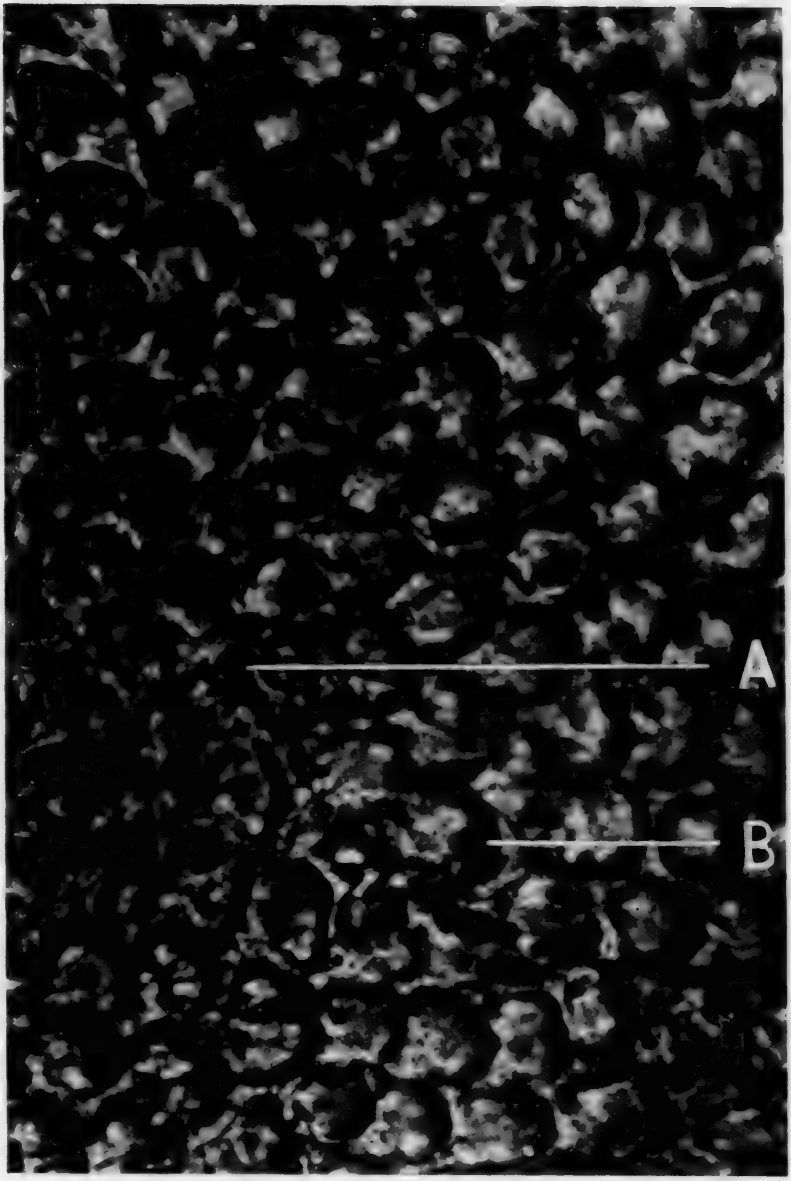

FIG. 64. Another cross section from the center of a spiral near the dento-enamel junction, showing, at *A* and *B*, unusually large canals. (See *fig.* 63.)

enamel caries is sometimes arrested. Is that arrest due to some change in the chemical contents of the saliva which inhibits the action of the bacteria or to some buffer condition set up in the substance of the enamel due to the circulation of a fluid in this tissue? These are questions which have not yet been answered.

Another question which the facts force upon us is this: to what extent is faulty structure a predisposing cause of decay? I should say, "Not in the least, unless the acid of decay is present." It is a mistake, as I long ago pointed out, to assume that the absence of

FIG. 65. Showing very defective formation of enamel on buccal surface of upper teeth of gorilla. Such a condition in human teeth would always be pronounced pathological, but it is not uncommon in animals, whose teeth never decay.

caries in the teeth of wild animals is due entirely or chiefly to more perfectly formed enamel than we find in the average human tooth. A few photographs of those animals which stand nearest to man will illustrate my point of view. *Figure 65* shows a buccal view of the cuspid, premolars, and molars of a large ape. You will see that the surface of the enamel is very rough, showing many grooves. It shows nothing of the fine lustre and polish which we regard as characteristic of the best human enamel. When we find enamel on human teeth resembling this, we say that the formation is very imperfect,

and we predict that such teeth will decay early and rapidly. *Figure 66* shows the masticating surface of one of these teeth. There are many more grooves here than we find on the masticating surfaces of human teeth, and yet these teeth do not decay. When we come to

FIG. 66. Masticating surface of molar of chimpanzee, ground through the sulci to show very imperfect formation and fusing of enamel.

examine sections of these teeth under the microscope, we frequently find evidence of imperfect calcification. Here are photographs of sections of enamel from the gorilla, the orang, and the chimpanzee, and in all of them there are areas of defective calcification (*figs. 67* and *68*). Faulty enamel does not necessarily mean decay, and the logical correlative of this statement is that the finest enamel that was ever formed will not resist the action of lactic acid as produced beneath bacterial plaques. There is nothing in this view which tends to minimize the great importance of the most perfect structure in enamel that can be produced, because the more imperfect the structure the more

FIG. 67. Imperfect formation of enamel in the larger apes

rapid will be the process of decay when the active or exciting cause is present.

With reference to the part which good habits and nutrition play in dental caries my view is this: the proper balance and optimum quantity of all the essential elements are most important for the preservation of the teeth. The greatest importance of this condition is during the period of gestation and the first seven years of life. This period determines the quality of the structure of enamel. But, as we have seen, that is not all that is required to maintain the integrity of the enamel throughout life. A well-balanced diet throughout life is probably necessary to the maintenance of that buffer condition, whatever that condition may be, which inhibits the action of the acid-producing

bacteria which are the active cause of decay. But even when all these food conditions are perfectly fulfilled, there will still be a wide variation in the liability of the teeth of different individuals to decay. There are other important conditions besides food elements which exert a powerful influence on nutrition. An examination of these

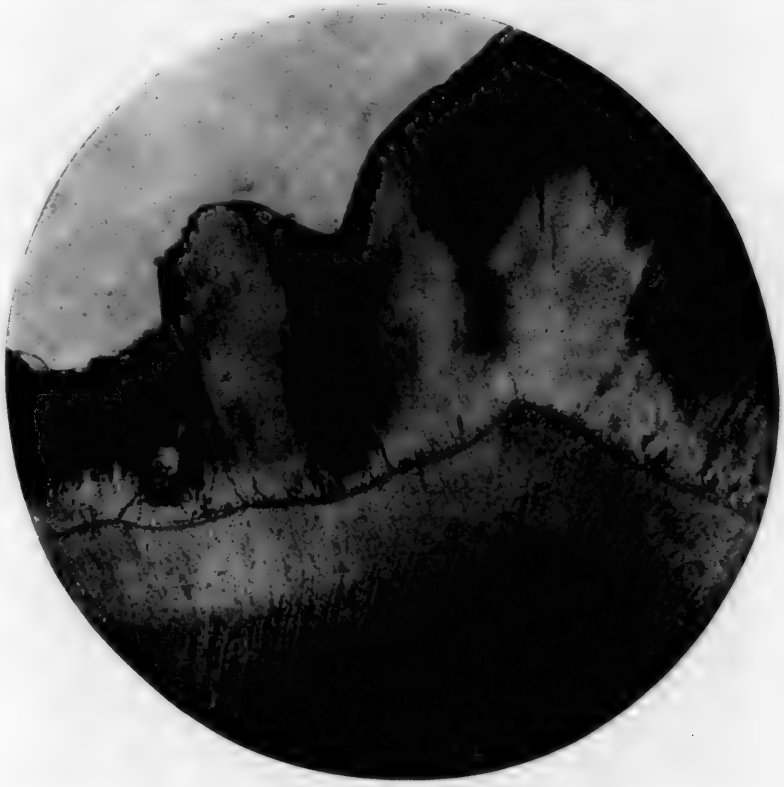

FIG. 68. A specimen of imperfect enamel from the orang

conditions is outside the province of this paper, but it will not be out of place to call attention to the fact, most noticeable and significant in remote country districts, that in large families which have been brought up on the same diet there is great variation in the liability of the teeth of the children to decay. And, as I have long noticed,

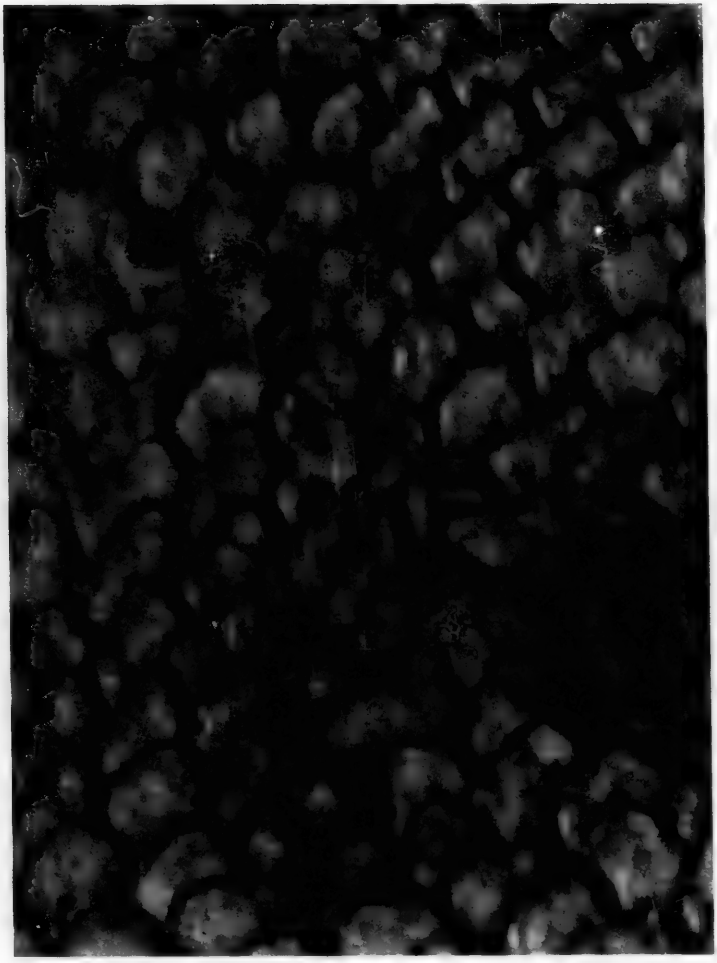

FIG. 69. Photograph of transverse section of the enamel of the Asiatic elephant, magnified nearly 3000 diameters. This photograph is interesting as showing the peculiar shape and arrangement of the enamel rods and the comparative absence of cement-substance. But it is introduced here especially to show that the sheaths of the enamel rods become deeply stained while the substance of the rod itself remains unstained, and that decalcification is not necessary to demonstrate this discrete substance.

the same thing is true of different families living in country districts where the food habits are practically identical. There are doubtless some very subtle influences at work, perhaps chiefly conditions of heredity and endocrine balance, which determine this question of immunity or non-immunity to dental caries.

There is just one more important phase of this problem to which I wish to call your attention. The work of Professor McCollum and others has shown us that defective bones and teeth may be the result of an unbalanced diet. That, of course, means defective organization of the whole body. But evidently every other tissue in the body except the enamel of the teeth may be restored to a normal condition. Every community in this country could probably produce instances of people who, having lost their teeth early in life, yet continued to live in health and vigor until a ripe old age was reached. Is not this another proof that all recuperative power lies in cell activity? From this point of view it would seem that the enamel has no recuperative power because its formative cells are completely lost with the eruption of the teeth.

We see, then, when our subject is viewed from all possible angles, that the outstanding fact seems to be that acid-forming bacteria are the active cause of caries of enamel. How to produce a *permanent* buffer condition in enamel, if such a thing be possible, is, therefore, our next great problem.

V. SUMMARY OF GENERAL CONCLUSIONS

The special new features in the normal and pathological histology of enamel brought out in this paper may be summarized as follows:

(1) The demonstration *without decalcification* of a sheath surrounding the enamel rod.

(2) This sheath is the first portion of the enamel to react to stains.

(3) This sheath appears to be in intimate relation with Nasmyth's membrane; in fact, firmly attached to and continuous with it.

(4) In normal, completely formed enamel this sheath stains evenly all around the rod.

(5) In mottled enamel and in the early stages of decay and, to some extent, in fully formed but unerupted teeth, *a portion only* of the sheath stains sharply and rapidly, the stained portion extending half or two-

thirds around the rod, thus showing in transverse sections a crescent or horseshoe form. This is an outstanding fact in the pathology of enamel, and one for which I have no explanation to offer at present.

The appearances of imperfect calcification in mottled enamel are of a very specific character. Three phases are clear in the illustrations which accompany this paper: (a) an abnormal condition of the sheaths of the enamel rods, which causes them to react to stains much more quickly than in normal enamel; (b) the presence in the cement-substance, and contiguous to and apparently involving the rod sheath, of numerous canals through which stains flow freely and quickly; (c) in the substance of the enamel rod numerous granules and spherical bodies are seen, which may indicate either abnormal chemical reactions or the arrest of those normal chemical processes which result in that complete fusion of substance and the resulting vitrified appearance which we see in perfectly formed enamel.

That the condition of mottled enamel is due to imperfectly calcified material is to a considerable extent demonstrated by the very similar appearances shown in fully formed but unerupted teeth in which the processes of calcification are not completed.

All stains which penetrate the enamel through the pulp chamber and the dentine enter by way of canals which, in human enamel at least, are always to be found in the center of the enamel whorls or spirals which constitute the characteristic arrangement of the enamel rods.

Caries of enamel is seen to begin with a change in the rod sheath, which renders it more sensitive to stain, and with the enlargement of canals which apparently exist in the most perfectly formed human enamel, and with the formation and enlargement of new canals either in the substance of, or contiguous to, the rod sheath.

The only rational explanation of these appearances that has yet been offered is that they are due to the action of bacterial products, formed beneath bacterial plaques, which products are known to possess the chemical power of dissolving enamel.

Certain premises have been inferred or assumed, and from these premises by processes of deductive reasoning a conclusion reached, that nutritive metabolic changes occur in enamel after eruption of the teeth. No microscopic evidence is available in confirmation of this opinion. All the facts in our possession point to the formative

period of enamel as the time when the quality of enamel is determined for life.

It is certain that all of the important facts concerning the effects of diet on enamel formation which recent experimental research has brought to light are mainly significant with reference to the period previous to the eruption of the teeth when the enamel is being formed.

Our present information concerning the structure of enamel and the processes of decay of this tissue would indicate the possibility of producing a buffer condition against the action of those bacterial products which are the active causes of caries by the external use on the enamel of some chemical substance which might enter and permanently remain in the canals and spaces which are found in all human enamel.

VI. ADDENDUM[4]

The foregoing paper, as may be seen in the first foot note, was read at Cincinnati, Ohio, on December 5, 1922. Interruption in the issuance of the numbers of this JOURNAL, in which I wished this paper to appear, has caused a somewhat lengthy delay in its publication.

Quite recently—on January 2, 1925, to be exact—my attention was called to an item in the London *Lancet* which referred to a paper, in the *British Dental Journal*, by Mr. H. C. Malleson, M. R. C. S., L. R. C. P., on the structure of human enamel as revealed in decalcified sections. This paper was read on April 9, 1924, before the London Metropolitan Branch of the British Dental Association. I looked up the issue of the *British Dental Journal* containing this paper and was delighted to find it a notable piece of work. As a considerable part of Mr. Malleson's paper deals with a subject that I have treated in the article herewith published, and as his paper was published before mine, it would seem almost discourteous in me to let the results of my own investigations appear without the foregoing explanation. Moreover, it gives me much pleasure to add my congratulations and felicitations to the many others which Mr. Malleson has received on his fine work. As Mr. Mummery very truly observed in his discussion of the paper, it opens up an entirely new conception of enamel structure.

[4] Added to the original manuscript on January 19, 1925.—(*Ed.*)

It is interesting to note in this connection that Dr. Charles F. W. Bödecker, formerly of Berlin but now Professor of Histology in the Dental School of Columbia University, succeeded, several years ago, in demonstrating an "organic" matrix in decalcified enamel by methods very similar to those described by Mr. Malleson. Working in my laboratory during the summer of 1923, Dr. Bödecker repeated his experiments and subsequently presented the results of this work before the International Association for Dental Research in New York.

I have decided to publish my own paper substantially as it was originally written and read, and to submit a few brief comments on the more recent work of Mr. Malleson, Dr. Bödecker and others as an addendum. The first thing to be said is that there is no longer any doubt whatever that the "organic" sheath which surrounds the enamel rods is albuminous and therefore protoplasmic in character. Prof. William J. Gies, head of the Biochemical Department of Columbia University, working in collaboration with Dr. Bödecker has made a thorough test of the undissolved matrix of some of Dr. Bödecker's specimens and found that it contains true protein matter. The first question that naturally arises, then, is this: what sort of "organic" matter is this, and have we any evidence as to its function in enamel? In this connection Mr. Malleson makes a very pertinent and significant suggestion. He says: "It may be asked, what is the nature of this cuticle? Is its structure the same as the translucent layer of Nasmyth's membrane and may not Von Ebner's statement that it is traceable into continuity with that membrane be correct?" I was not aware that Von Ebner had made this observation, but I believe that the illustration which I show as *fig. 56* amply confirms this view. It is, I think, generally believed that Nasmyth's membrane belongs in the keratin group of substances, of which cuticle, horn, hair, hoof, claws, nails, feathers, etc. are chiefly composed. Prof. Gies has called my attention to the fact that various forms of these epidermal substances, especially cuticle, possess considerable elasticity.

My suggestion is that the sheaths of the enamel rods and Nasmyth's membrane are similar in physical character and chemical composition, and that it is the elastic property of these sheaths which preserves the

very brittle enamel from destruction during the stress of the mastication of hard substances. If the enamel throughout were equally hard and brittle we may reasonably conclude that, especially with the rodents and carnivorous animals, it would simply crack and go to pieces with such usage as these teeth get. This view makes the sheath of the enamel rod one of the most important features in this tissue.

But there is at present a growing belief with some that nutritional changes, true metabolic activity, take place in enamel through the medium of this "organic" matter. If we wish to apply the term "metabolism" to inorganic, chemical changes, then we may say that theoretically this is possible. Some are doing this. Dr. Percy Howe, for example, speaks in a recently published paper of "inorganic metabolism." But we must not forget the saying of one of the great master minds that the first essential of sound thinking is a clear perception of distinctions and differences. Many chemical actions and reactions are included in cell metabolism, but this great distinction and difference between inorganic chemical action and cell metabolism remains—one is the characteristic of living things and the other of dead things. Enamel is a product of cell metabolism, but when its production is completed the cells which produced it disappear. That is not true of any other animal tissue. With all other organs and tissues the formative cells remain, hence the possibility of repair and regeneration. Any material for the repair or regeneration of enamel from an internal source must come through the dentine-forming cells. But dentine-forming cells produce dentine, not enamel. Have we any evidence that there is ever a deposit of dentine in enamel? Have we any evidence that there is any sort of an amorphous deposit from the dental pulp of lime salts in enamel after the formation of enamel is completed? None whatever, as far as I know.

Let us keep our feet on the solid ground of facts. Even the most insecure of hypotheses must lay claim to some facts to justify its existence, and I repeat that I know of no fact which warrants the claim that anything in the nature of true regenerative changes takes place in enamel after the disappearance of the ameloblasts. I should be greatly delighted to hear that such a fact had been discovered. The day on which such a discovery may be made will indeed be a momentous one for humanity. But for the present I think the dental profes-

sion need have no fear that Othello's occupation will be gone on the assumption that nature has discovered a way to anticipate the results of artificial professional skill. And so I do not look upon this discovery of "organic" matter in enamel as giving the slightest warrant for the opinion that real, nutritional, metabolic changes are taking place in enamel.

This conclusion is not in accordance with the hopes which I expressed in the opening of the paper on "*unsolved problems in dental histology*," which I read, in 1922, before the International Association for Dental Research.[6] I had hoped to find some evidence of reconsolidation of enamel after eruption of the teeth. After four years of the most painstaking search, during which more than one thousand photographs similar to those illustrating this paper have been made, no such evidence has been discovered. It seemed to me, when I found the readiness with which enamel could be stained, that the way was open for the deposit of calcific material in defective, porous enamel, not as true enamel but in a form which would increase its power of resistance. But I find that teeth in the mouths of elderly people, teeth which have resisted the powers of decay for a lifetime, often have an open, porous structure of enamel which stains quickly penetrate. I find the same condition in the teeth of animals, where caries is never found, but nowhere can I find any evidence that these canals, which greatly favor rapidity in the progress of caries *when the exciting causes of decay are present*, are ever consolidated or filled up.

And there is another line of evidence that is almost conclusive that there is no improvement in the quality of enamel in erupted teeth even by "inorganic metabolism." Some eight years ago Prof. Gies repeatedly injected Trypan blue into the abdominal cavity of a puppy, at short intervals beginning several days after birth and while the teeth were in process of formation. All the tissues of the body were deeply stained. As the teeth erupted it was seen that all the enamel was deeply stained. In another puppy, similarly treated during a later period of enamel formation, the enamel which had been completed before the first injection remained unstained. The stain rapidly disappeared from all the tissues *except the enamel of the teeth*, and now, after eight years, that stain remains in the enamel without change. I

[6] Williams: *Journal of Dental Research*, 1923, v, p. 27.

have repeated this experiment with quite a number of animals, and always with the same results. Do these observations not force the conclusion upon us that there is no vital circulation in enamel?

I think it quite time to call on the gentlemen who hold and vigorously advocate contrary views to bring forward, not mere opinions, not assertions that because *this* is so *therefore that must* be so, but demonstrated facts—such facts as only the microscope or very ably conducted chemical analyses are able to reveal—that there has been a deposit of secondary enamel, or any sort of calcific deposit, in places where this tissue was defective.

Circulation of fluid and albuminous matter of a sort probably occurs in enamel, presumably in all teeth; but, if the presence of such fluid and albuminous matter contributes anything to improvement of the quality of enamel, then why does not the quality of all enamel in all teeth gradually improve with age? If in reply the conjecture is put forward that the unknown conditions may not always be favorable, then I have to say in rejoinder that we can see and study, in all the different phases which are presented, the degenerative changes which occur in bone and dentine, as the result of a deficient or unbalanced diet. We can watch the destructive machinery, so to say, at work. But what machinery or what destructive agents, apart from bacteria or their products, have ever been discovered engaged in the business of causing degenerative changes in enamel?

Whatever, therefore, the future may reveal, I know of no facts at the present time on which one may base an intelligent opinion that there are either regenerative or degenerative vital metabolic activities in the enamel of fully erupted teeth.

The demonstration through experiments on animals of the hygienic value of rational food habits and a scientifically balanced diet, for the body in its entirety, is one of the greatest contributions to human welfare of the present time. But continued insistence on a theoretical application of dietary criteria to the enamel of fully erupted teeth, for which no proof is forthcoming, has the effect of drawing attention away from the main significance of the work and thereby losing something of the appreciation of its real value.

There are now a large number of facts which stand in confirmation of the statement which I made many years ago that the observed dif-

ferences in the liability of teeth to decay are due not primarily to differences in structure but to differences in conditions of environment. Of course, when the environing conditions favorable to caries are present, teeth of defective structure will decay more rapidly than those of better formation.

Mr. Malleson says in his paper: "Further investigation of caries should show by which path the destruction of enamel takes place;" and he asks: "Is it via prism, interprismatic substance or prism cuticle?" I think it will be clearly seen that the illustrations accompanying this article, as shown in *figs. 51* and *52*, answer Mr. Malleson's query. Decay always begins on the inner side of the rod sheath, or what he calls the "prism cuticle." The appearances indicate that the acid of decay passes down along the inner wall of the rod sheath and attacks the outer part of the rods. We see that this deeply stained area around the enamel rod begins to enlarge. A canal is formed and the area of this canal increases and encroaches more and more on the substance of the rod and the inter-rod cement-substance. As the canals continue to increase in size they become confluent, and at last the tissue breaks down and we have the commencement of a cavity. Can any one imagine any other cause for such appearances as I have shown in the illustrations which accompany the foregoing paper than the action of bacteria? Dr. Percy Howe and others are claiming that caries is the result of metabolic changes in the substance of the enamel. I freely accept the *evidence* he presents, as to facts, of the results of all his valuable work, but the evidence presented does not warrant some of his conclusions. If metabolic changes in enamel were the chief cause of caries, then cavities might form internally or at any part of the tooth, and they would be just as likely to form on the labial surface of a tooth as elsewhere. But we all know that this is not the case. Cavities never form except at such localities as bacterial plaques may form and act undisturbed. I have, from time to time, presented a large array of facts in support of the position that caries of enamel is due to bacterial action. No one, as far as I know, has ever succeeded in controverting any of these facts. All evidence in opposition to this view has been purely negative in character.

Mr. Malleson brings forward another point which I am glad to note.

He says: "It has been suggested that the inter-columnar bridges correspond to the prickle processes seen between epithelial cells and presumably so between the ameloblasts. If this is correct it would appear as if the ameloblast itself is calcified." This is precisely the view that I have been advocating for more than twenty-five years. And, as may be seen in many of the illustrations accompanying this paper, it is not only the inter-rod bridges which support this view, but everywhere in the enamel rod are seen evidences of calcified protoplasmic structure.

There is one more suggestion in Mr. Malleson's paper on which I will comment, and that is the difference of opinion that has been expressed as to the presence of an inter-rod or cement-substance in enamel. This substance is certainly present and is clearly seen in many of my photographs. (See *figs. 33, 52* and *62*). But the amount of this substance varies greatly in different specimens. In most forms of enamel the rods are irregularly round. Here, in the nature of the arrangement, there must be considerable cement-substance. But in some animals, the Asiatic elephant (*fig. 69*), for example, the rods are pressed so closely together as to leave but little space for cement-substance. Such a condition is occasionally seen in human teeth.

It will be seen that on most points I am in entire agreement with the conclusions in Mr. Malleson's paper, and I hail with much pleasure the appearance of his admirable and able work.